Sal I Bam H I

| |

---------- - pUC 19

|

Xba I

1 **ATGGAGTCAA AGTTTGCTCA CATCATTGTT TTCTTTCTTC TTGCAACTTC**

original sequence - ag a

51 **CTTTGAAACT CTCTTGGCAC** GAAAAGAAAG Tgatggacca gagatcttag

mutagenic primer

101 aacTTCAAAA GGAATTTGAA TGCAATGGAA AACAAAGGTG GCCAGAACTT

151 ATTGGTGTAC CAACAAAGCT TGCTAAGGGG ATAATTGAGA AGGAAAATTC

201 ACTCATAACT AATGTTCAGA TACTACTGAA TGGTTCTCCA GTCACAATGG

251 ATTATCGTTG TAATCGAGTT CGTCTTTTTG ATAACATTTT GGGTGATGTT

301 GTACAAATTC CTAGGGTGGC TTAA

Figure 1

1 GAATTCCGCA AGGAgcacac ccggctgtcc acctgCTGCA GAG**ATGGTGC**

upstream primer

51 **ACGCAACCTC CCCGCTGCTG CTGCTGCTGC TGCTCAGCCT GGCTCTGGTg**

cc t original sequence

101 gctcccggga tccctgccag AAAGTGCTCG CTGACTGGGA AATGGACCAA

mutagenic primer

151 CGATCTGGGC TCCAACATGA CCATCGGGGC TGTGAACAGC AGAGGTGAAT

201 TCACAGGCAC CTACATCACA GCCGTAACAG CCACATCAAA TGAGATCAAA

251 GAGTCACCAC TGCATGGGAC ACAAAACACC ATCAACAAGA GGACCCAGCC

301 CACCTTTGGC TTCACCGTCA ATTGGAAGTT TTCAGAGTCC ACCACTGTCT

351 TCACGGGCCA GTGCTTCATA GACAGGAATG GGAAGGAGGT CCTGAAGACC

401 ATGTGGCTGC TGCGGTCAAG TGTTAATGAC ATTGGTGATG ACTGGAAAGC

451 TACCAGGGTC GGCATCAACA TCTTCACTCG CCTGCGCACA CAGAAGGAG**T**

501 **GA**GGATGGCC CCGCAAAGCC AGCAACAATG CCGGAGTGCT GACACTGCTT

↓ **Hind III**

551 GTGATATTCC TCCCCAATAA AGCTTG

Figure 2

EcoR I

```
  1  GAATTCGCAT ATGGCTGAAG CTGGTATCAC CGGTACTTGG TACAACCAGC
 51  TGGGGTCTAC CTTCATCGTT ACCGCTGGTG CTGACGGTGC ACTGACCGGT
101  ACTTACGAAA GCGCTGTTGG TAACGCTGAA AGCCGTTATG TTCTGACCGG
151  TCGTTACGAC TCTGCTCCGG CTACCGACGG TTCTGGTACT GCTCTGGGTT
201  GGACCGTTGC TTGGAAAAAC AACTACCGTA ACGCTCACTC TGCTACCACC
251  TGGTCTGGCC AGTACGTTGG TGGTGCTGAA GCTCGTATCA ACACCCAGTG
301  GCTGCTGACC TCTGGTACCA CCGAAGCTAA CGCTTGGAAA TCTACCCTGG
351  TTGGTCACGA CACGTTCACC AAAGTTAAAC CGTCTGCTGC TTCTATCTAGA
                                                  Xba I
```

Figure 3

Sal I altered Bam H I*

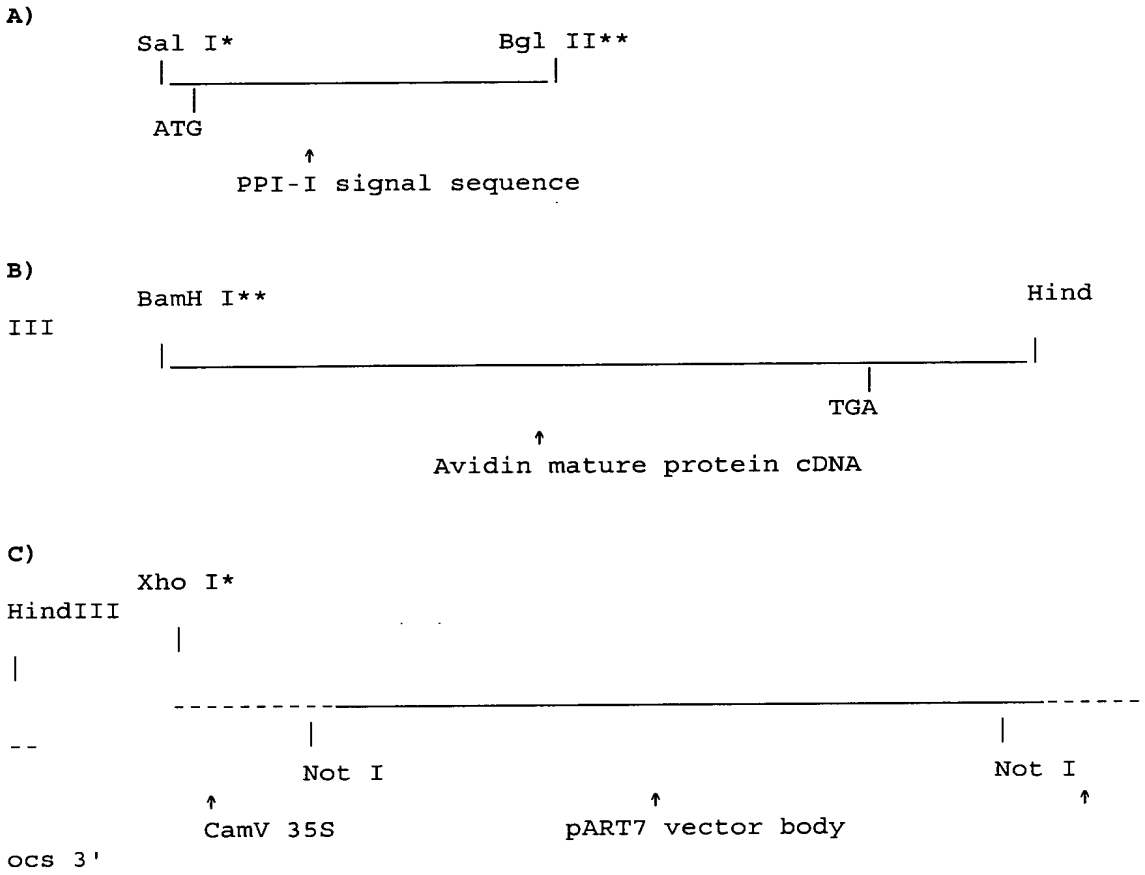
```
   |    |
---------- - pUC 19

     |
   Xba I
```

```
  1  ATGGATGTTC ACAAGGAAGT TAATTTCGTT GCTTACCTAC TAATTGTTCT
 51  TGGTAAGATT TTCCTTTACT CCTTTGTTTT AAAAAATAAA AAAACAAAAA
101  AAATCTTGGT TTATACATAT ATATACACAC AAGTAGTTTT ATTTTTTTCC
151  TTTATATTAT ATTTGTTGTA GGAATATTTC TACTTGTTAG CGTGGTGGAA
201  CATGTTGATG CGAAGATCTG TACTAAAGAA TGTGGTAATC TTGGGTTTGG
251  GATATGCCCA CGTTCAGAAG GAAGTCCGAA AAATCCCATA TGCATCAATT
301  GTTGCTCAGG CTATAAGGGT TGTAATTATT ATAGTGTTTT CGGGAGATTT
351  ATTTGCGAAG GAGAATCTGA CCTAAAAAAC CCAAAAGCTT GCCCCCTAAA
401  TTGTGATACA AATATTGCCT ATTCAAGATG CCCCCATTCA GAAGGAAAAT
451  CGCTAATTTA TCCCACCGGA TGTACCACAT GTTGCACAGG GTACAAGGGT
501  TGCTACTATT TCGGTAAAAA TGGCAAGTTT GTATGCGAAG GAGAGAGTGA
551  TGAACCCAAG GCAAATATGT ACCCTGCAAT GTGA
```

* result of PCR error during isolation of the PPI-II sequence

Figure 4



*** compatible cohesive ends**
**** compatible cohesive ends**

Figure 5

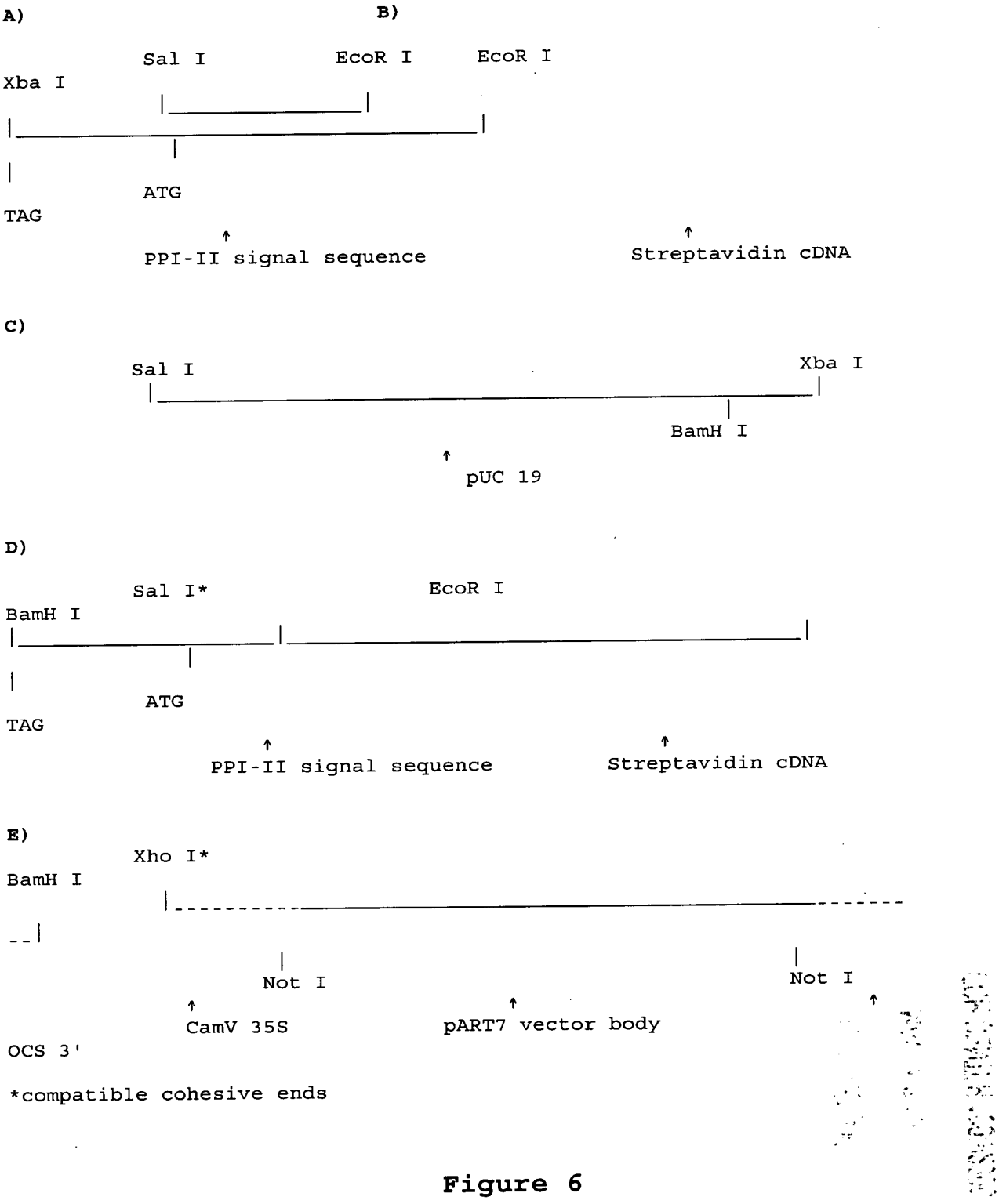
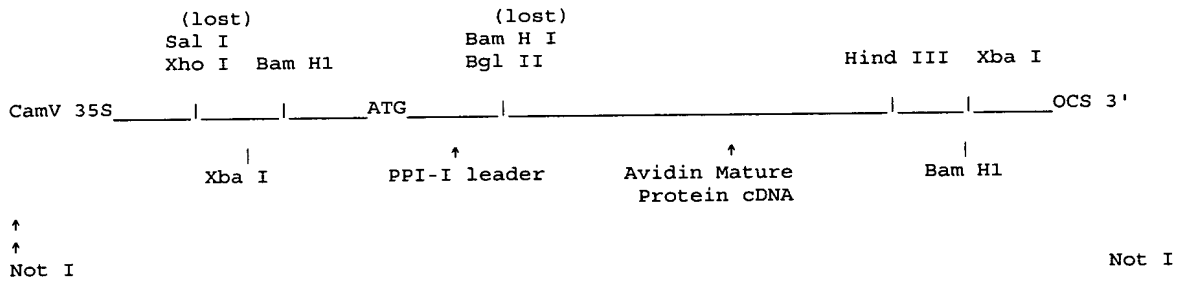


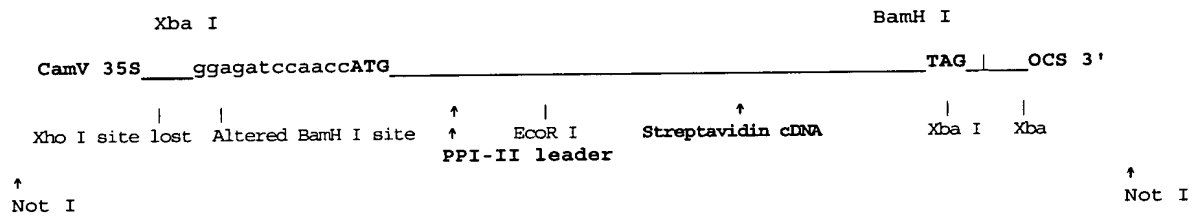
Figure 6

A)



pART27

B)



pART27

Figure 7

A)

```
   1  ATGGAGTCAA AGTTTGCTCA CATCATTGTT TTCTTTCTTC TTGCAACTCC
  51  CTTTGAAACT CTCTTGGCAC GAAAAGAAAG TGATGGACCA GAGATCCCTG
 101  CCAGAAAGTG CTCGCTGACT GGGAAATGGA CCAACGATCT GGGCTCCAAC
 151  ATGACCATCG GGGCTGTGAA CAGCAGAGGT GAATTCACAG GCACCTACAT
 201  CACAGCCGTA ACAGCCACAT CAAATGAGAT CAAAGAGTCA CCATTGCATG
 251  GGACACAAAA CACCATCAAC AAGAGGACCC AGCCCACCTT TGGCTTCACC
 301  GTCAATTGGA AGTTTTCAGA GTCCACCACT GTCTTCACGG GCCAGTGCTT
 351  CATAGACAGG AATGGGAAGG AGGTCCTGAA GACCATGTGG CTGCTGCGGT
 401  CAAGTGTTAA TGACATTGGT GATGACTGGA AAGCTACCAG GGTCGGCATC
 451  AACATCTTCA CTCGCCTGCG CACACAGAAG GAGTGA
```

B)

cleavage site ↓

```
   1  MESKFAHIIV FFLLATPFET LLARKESDGP EipARKCSLT GKWTNDLGSN
  51  MTIGAVNSRG EFTGTYITAV TATSNEIKES PLHGTQNTIN KRTQPTFGFT
 101  VNWKFSESTT VFTGQCFIDR NGKEVLKTMW LLRSSVNDIG DDWKATRVGI
 151  NIFTRLRTQK E*
```

Figure 8

A)

```
  1  ATGGATGTTC ACAAGGAAGT TAATTTCGTT GCTTACCTAC TAATTGTTCT
 51  TGGTAAGATT TTCCTTTACT CCTTTGTTTT AAAAAATAAA AAAACAAAAA
101  AAATCTTGGT TTATACATAT ATATACACAC AAGTAGTTTT ATTTTTTTCC
151  TTTATATTAT ATTTGTTGTA GGAATATTTC TACTTGTTAG CGTGGTGGAA
201  CATGTTGATG CGAAGATCTG TACTAAGAAT TCGCATATGG CTGAAGCTGG
251  TATCACCGGT ACTTGGTACA ACCAGCTGGG GTCTACCTTC ATCGTTACCG
301  CTGGTGCTGA CGGTGCACTG ACCGGTACTT ACGAAAGCGC TGTTGGTAAC
351  GCTGAAAGCC GTTATGTTCT GACCGGTCGT TACGACTCTG CTCCGGCTAC
401  CGACGGTTCT GGTACTGCTC TGGGTTGGAC CGTTGCTTGG AAAAACAACT
451  ACCGTAACGC TCACTCTGCT ACCACCTGGT CTGGCCAGTA CGTTGGTGGT
501  GCTGAAGCTC GTATCAACAC CCAGTGGCTG CTGACCTCTG GTACCACCGA
551  AGCTAACGCT TGGAAATCTA CCCTGGTTGG TCACNACACG TTCACCAAAG
601  TTAAACCGTC TGCTGCTTCT ATCTAG
```

B)

cleavage site ↓

```
  1  MDVHKEVNFV AYLLIVLGIF LLVSVVEHVD AKICTKnshM AEAGITGTWY
 51  NQLGSTFIVT AGADGALTGT YESAVGNAES RYVLTGRYDS APATDGSGTA
101  LGWTVAWKNN YRNAHSATTW SGQYVGGAEA RINTQWLLTS GTTEANAWKS
151  TLVGHDTFTK VKPSAASI*
```

Figure 9

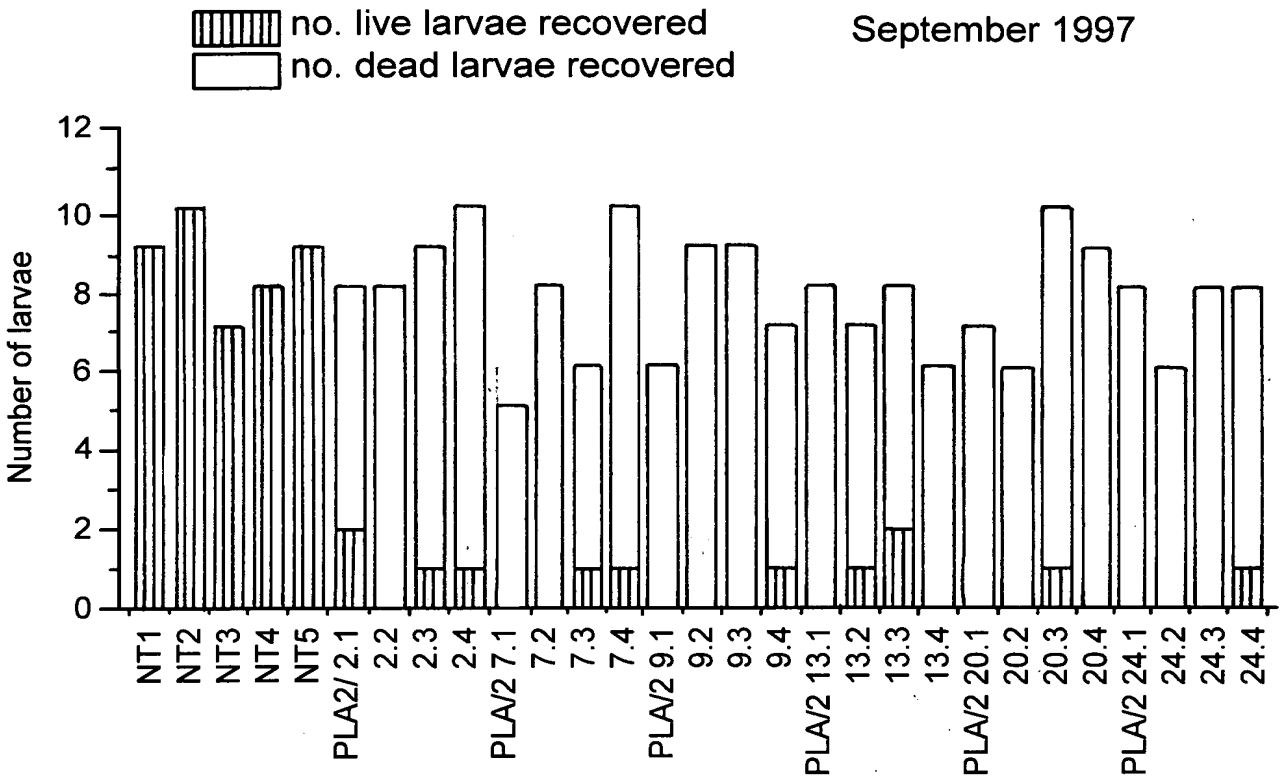


FIG. 10

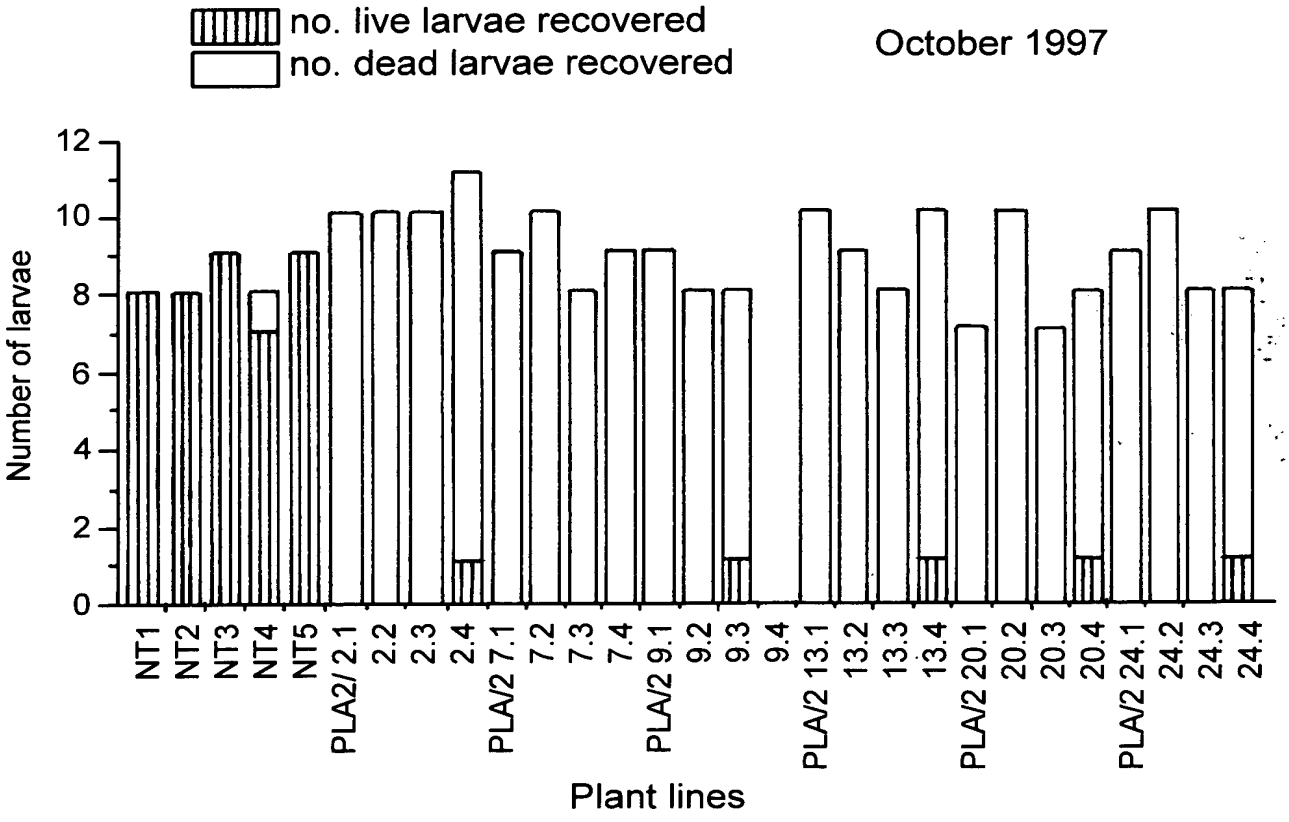


FIG. 11

A)

```
   1  CCCTCCGTCC CCGCCGGGCA ACAACTAGGG AGTATTTTTC GTGTCTCACA
  51  TGCGCAAGAT CGTCGTTGCA GCCATCGCCG TTTCCCTGAC CACGGTCTCG
 101  ATTACGGCCA GCGCTTCGGC AGACCCCTCC AAGGACTCGA AGGCCCAGGT
 151  CTCGGCCGCC GAGGCCGGCA TCACCGGCAC CTGGTACAAC CAGCTCGGCT
 201  CGACCTTCAT CGTGACCGCG GGCGCCGACG GCGCCCTGAC CGGAACCTAC
 251  GAGTCGGCCG TCGGCAACGC CGAGAGCCGC TACGTCCTGA CCGGTCGTTA
 301  CGACAGCGCC CCGGCCACCG ACGGCAGCGG CACCGCCCTC GGTTGGACGG
 351  TGGCCTGGAA GAATAACTAC CGCAACGCCC ACTCCGCGAC CACGTGGAGC
 401  GGCCAGTACG TCGGCGGCGC CGAGGCGAGG ATCAACACCC AGTGGCTGCT
 451  GACCTCCGGC ACCACCGAGG CCAACGCCTG GAAGTCCACG CTGGTCGGCC
 501  ACGACACCTT CACCAAGGTG AAGCCGTCCG CCGCCTCCAT CGACGCGGCG
 551  AAGAAGGCCG GCGTCAACAA CGGCAACCCG CTCGACGCCG TTCAGCAGTA
 601  GTCGCGTCCC GGCACCGGCG GGTGCCGGGA CCTCGGCC
```

B)

```
   1  MRKIVVAAIA VSLTTVSITA SASADPSKDS KAQVSAAEAG ITGTWYNQLG
  51  STFIVTAGAD GALTGTYESA VGNAESRYVL TGRYDSAPAT DGSGTALGWT
 101  VAWKNNYRNA HSATTWSGQY VGGAEARINT QWLLTSGTTE ANAWKSTLVG
 151  HDTFTKVKPS AASIDAAKKA GVNNGNPLDA VQQ
```

Figure 12

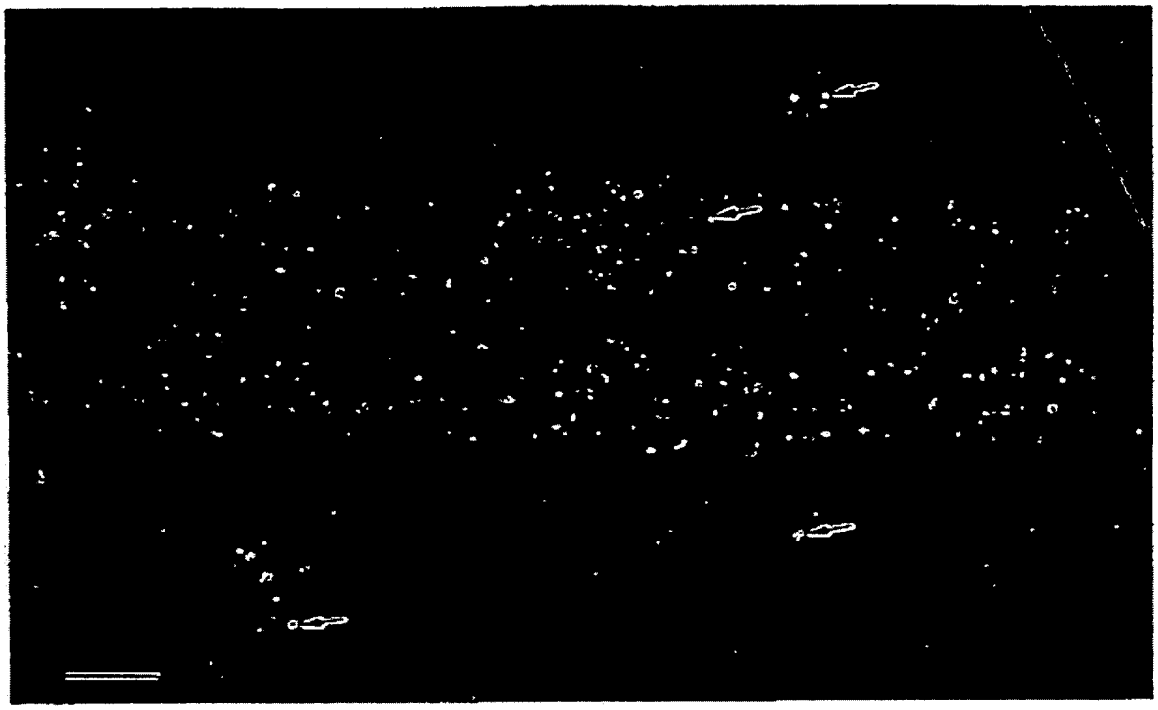


Figure 13

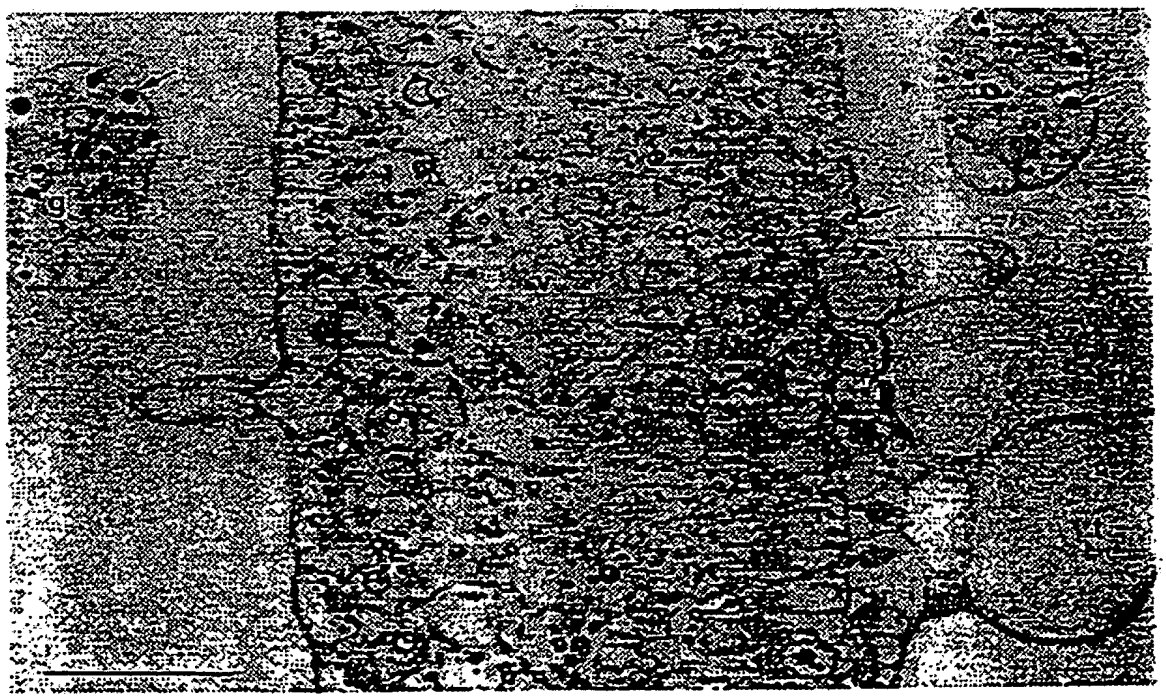


Figure 14

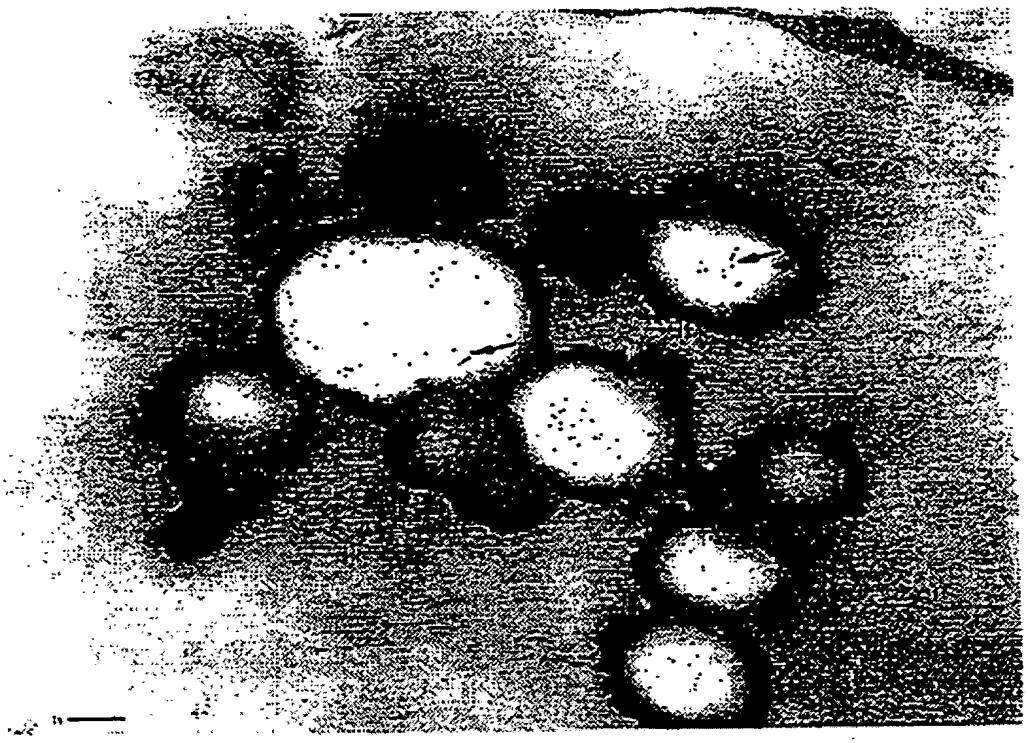


Figure 15

Figure 16

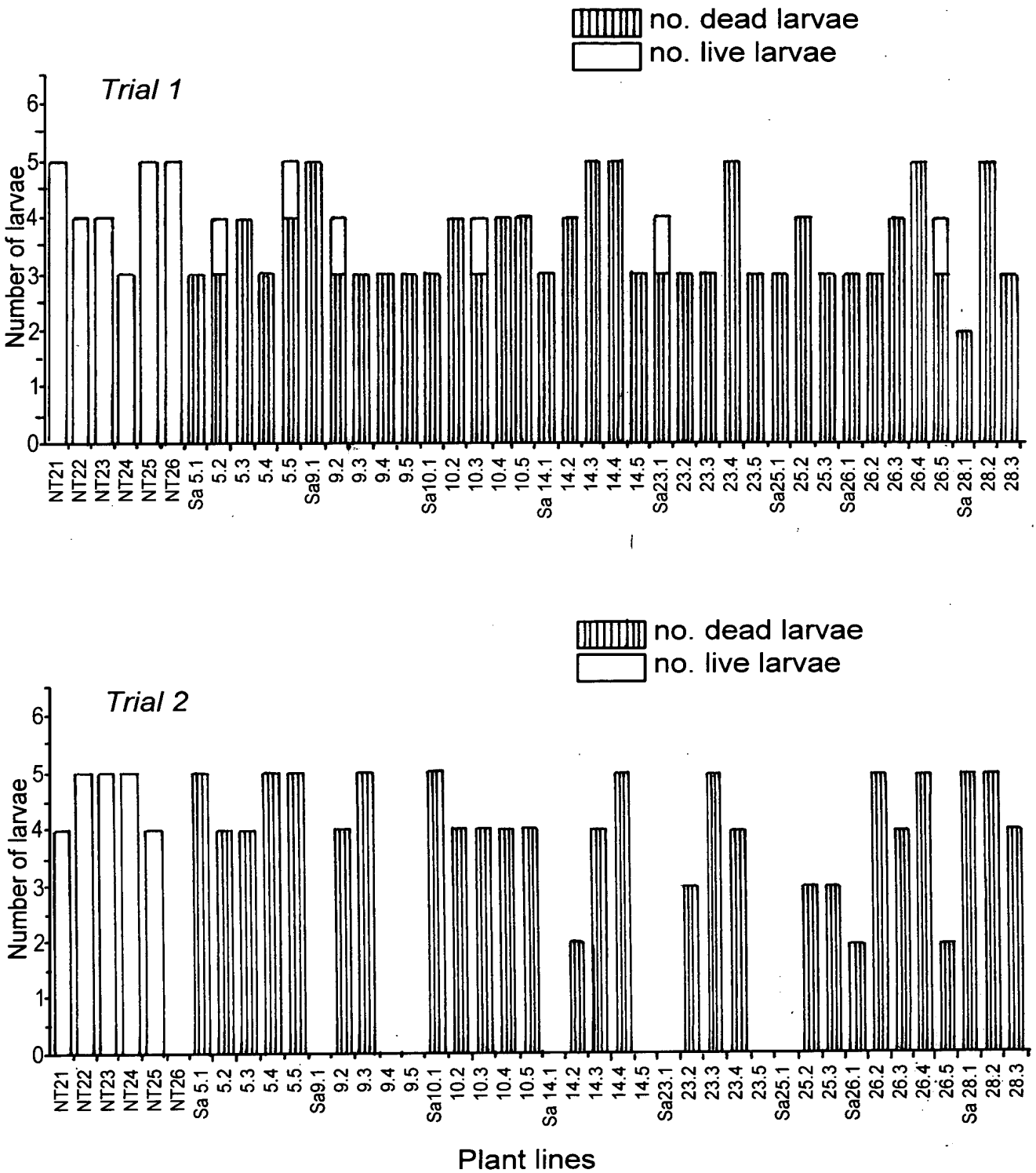


FIG. 17

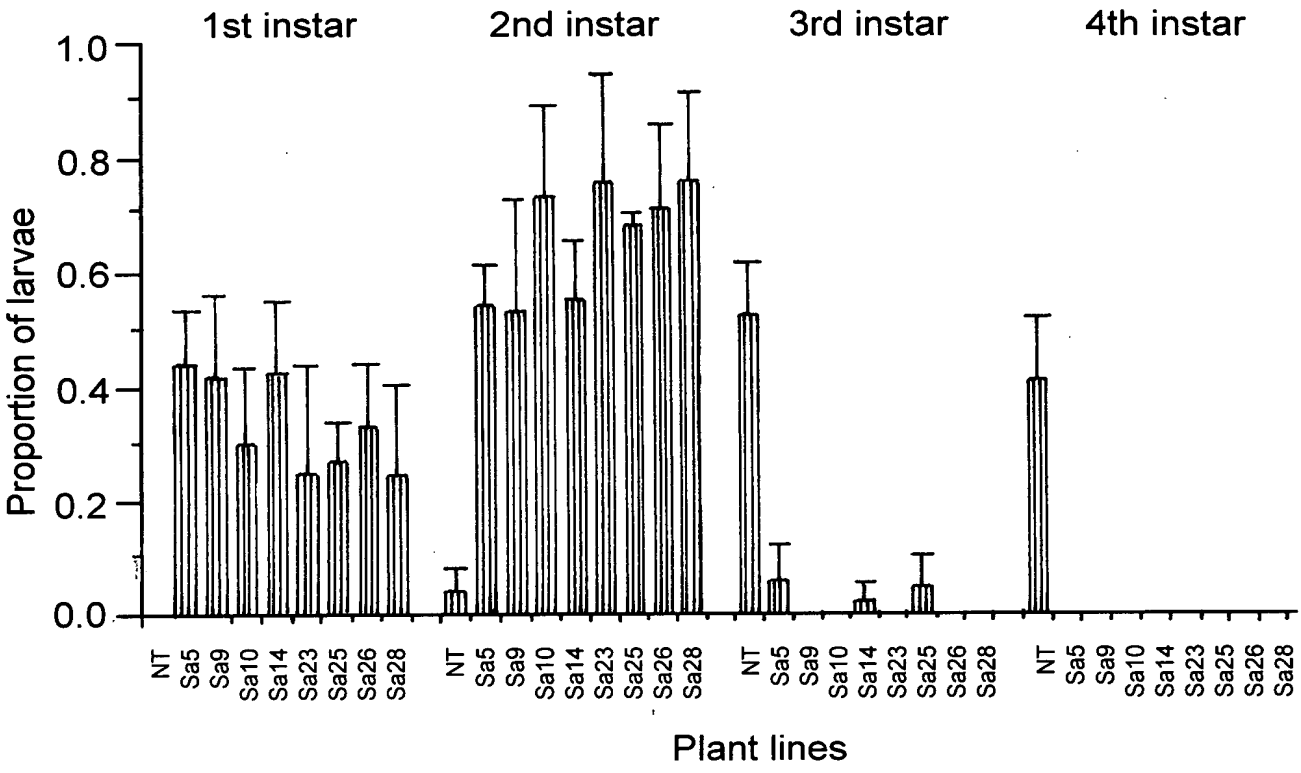


FIG. 18

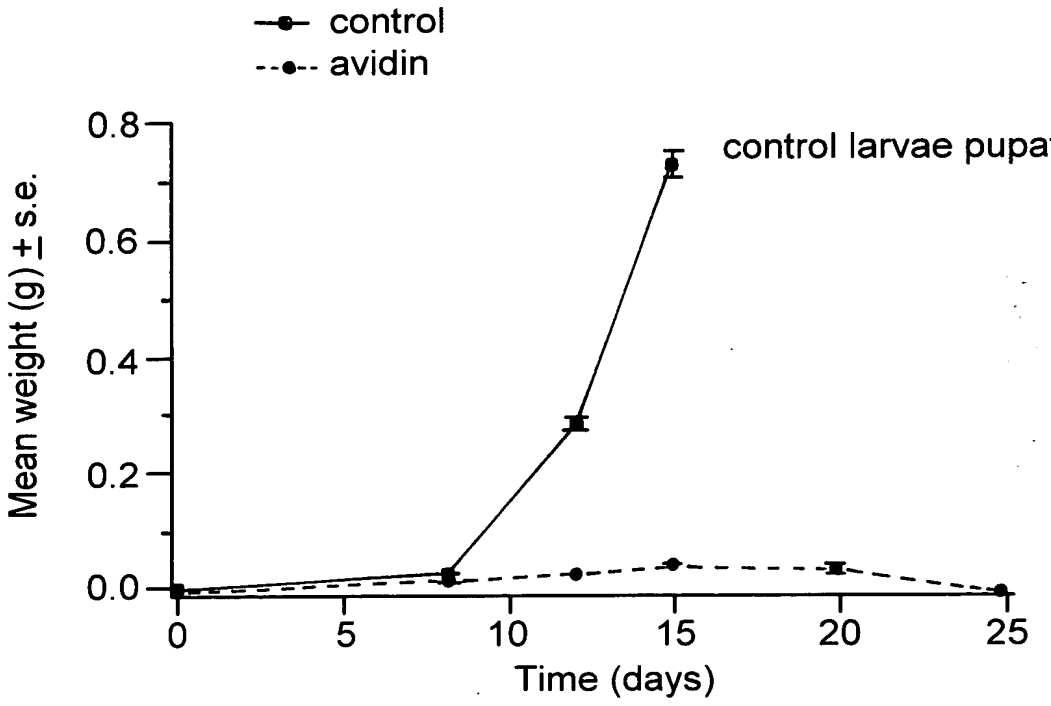


FIG. 19A

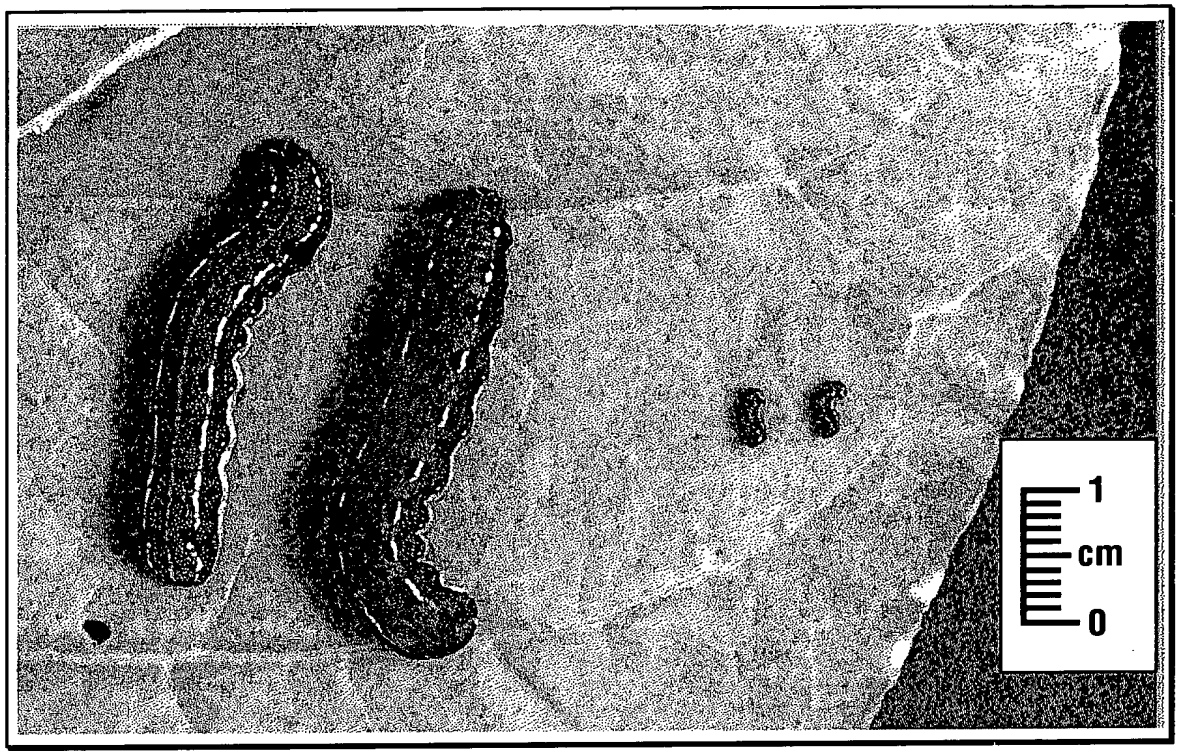


Figure 19B

Figure 19C

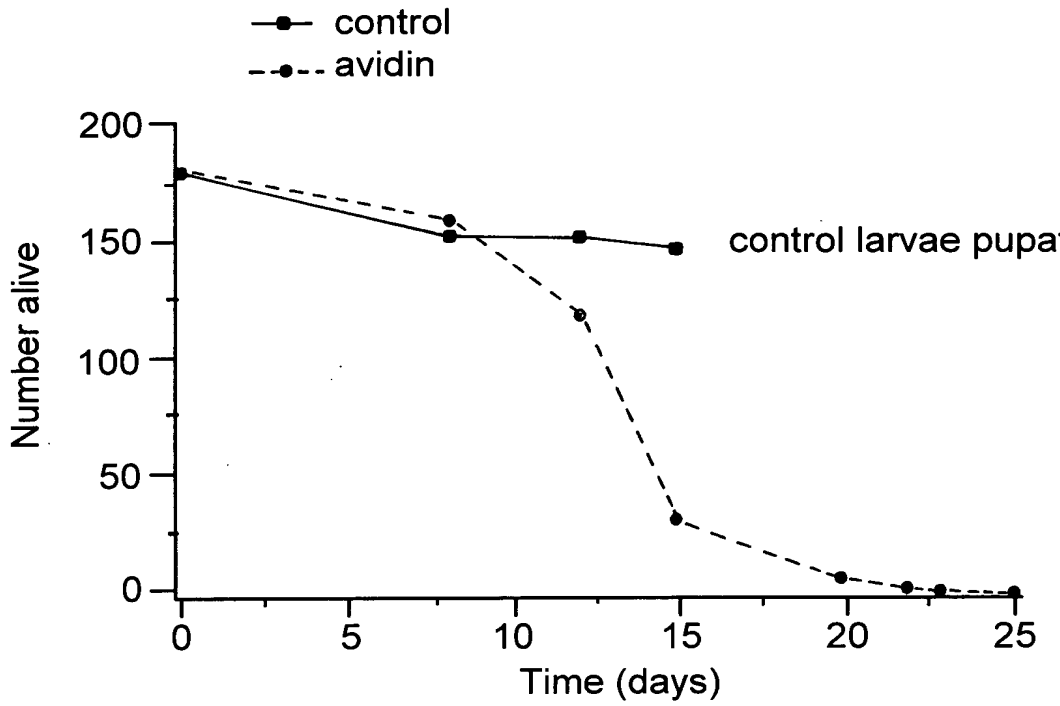


FIG. 20

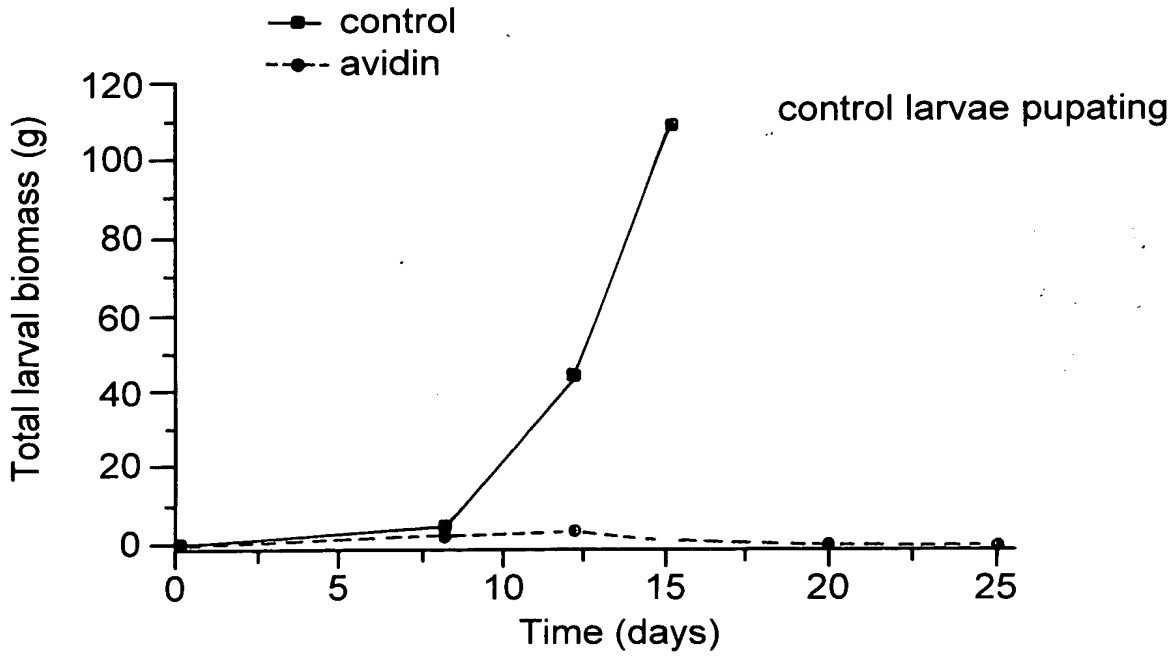


FIG. 21

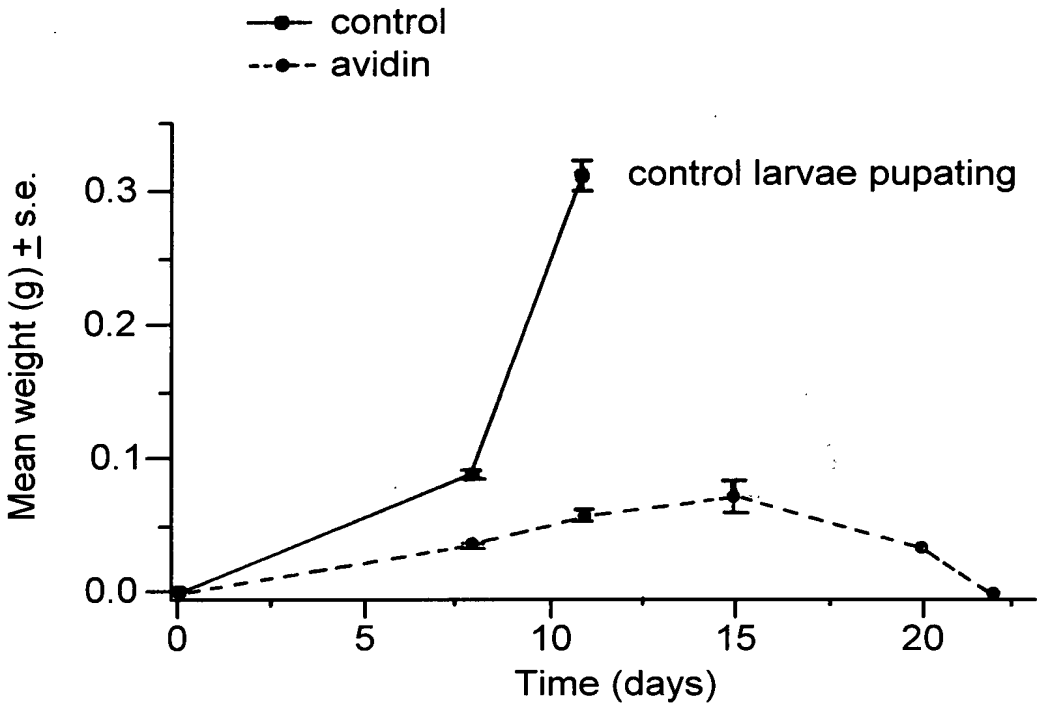


FIG. 22A

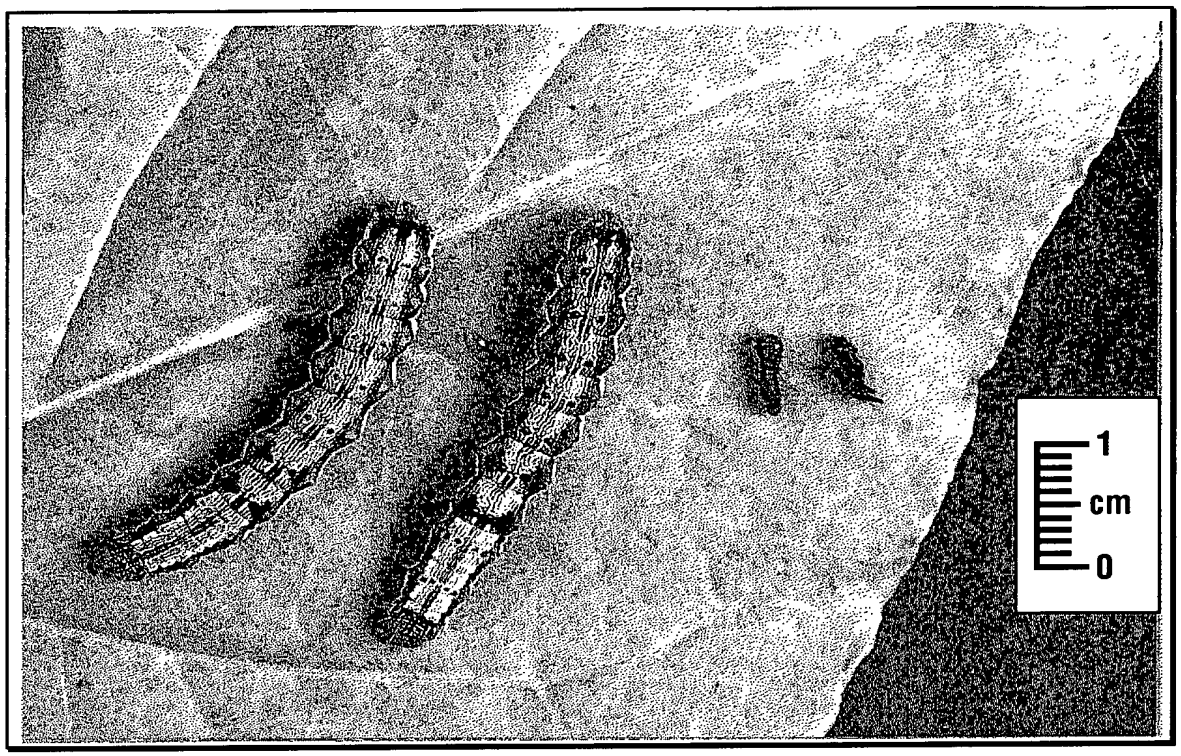


Figure 22B

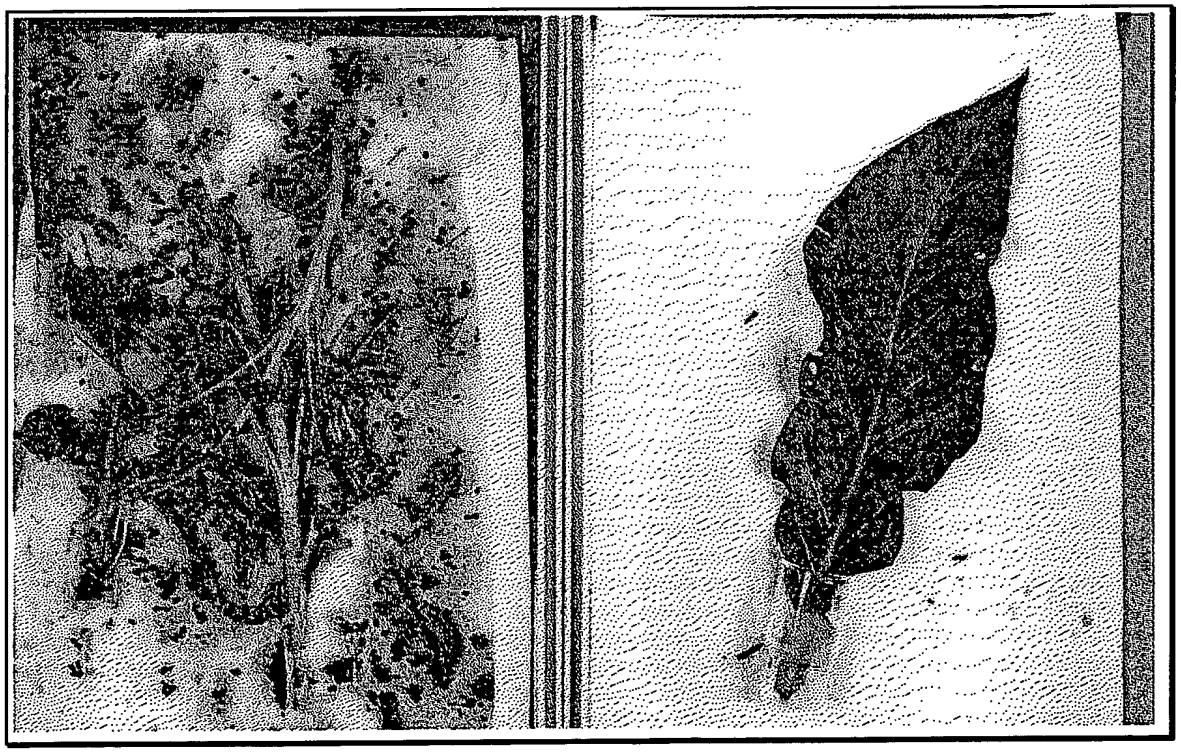


Figure 22C

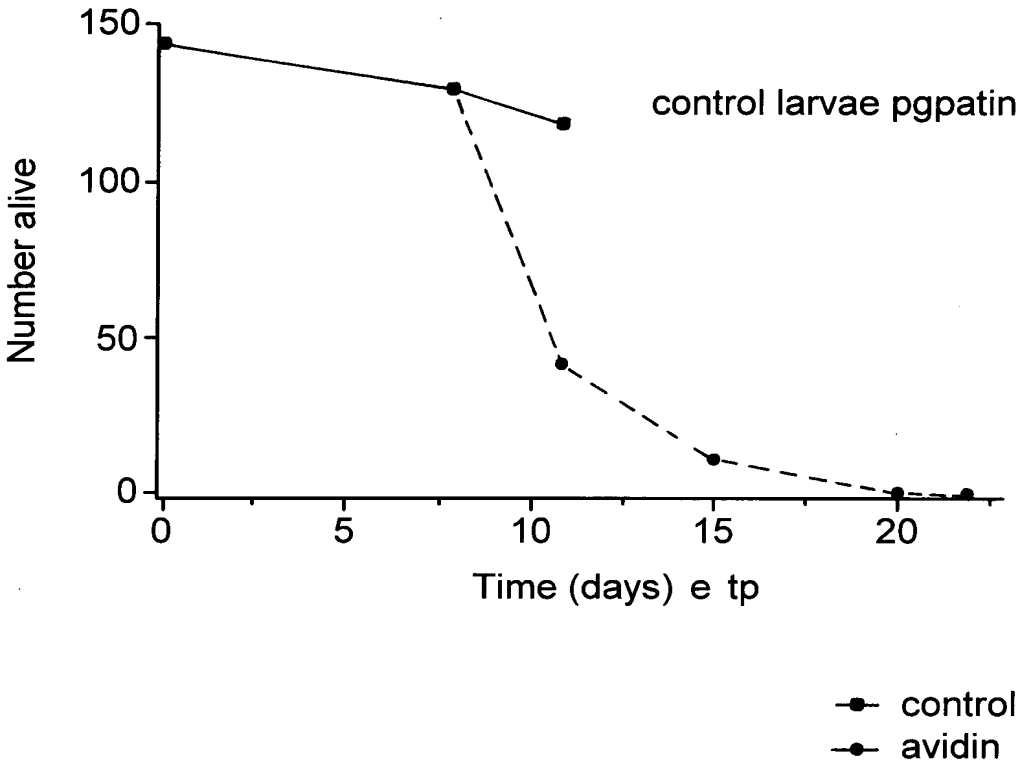


FIG. 23

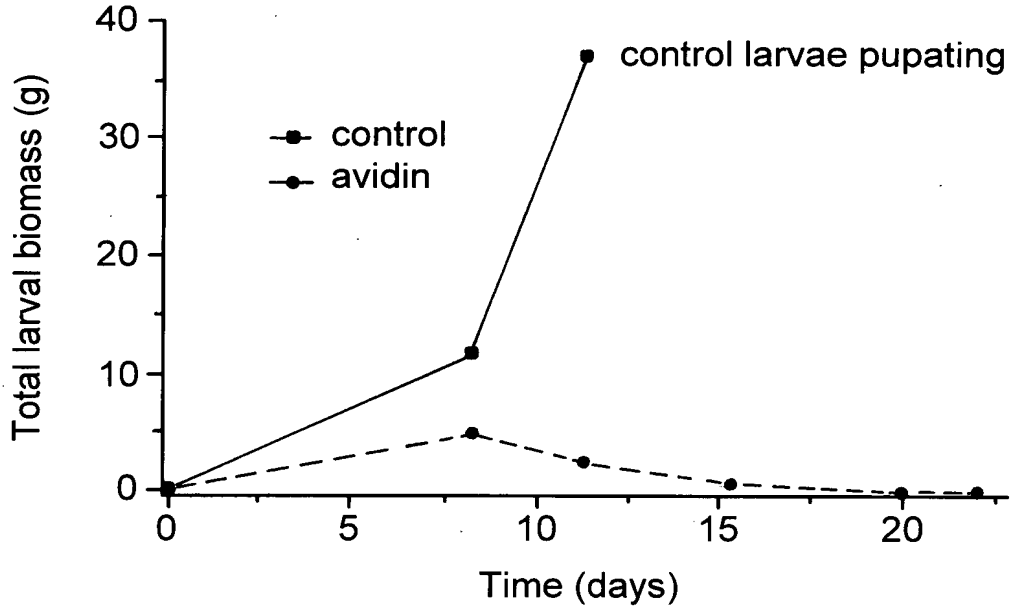


FIG. 24

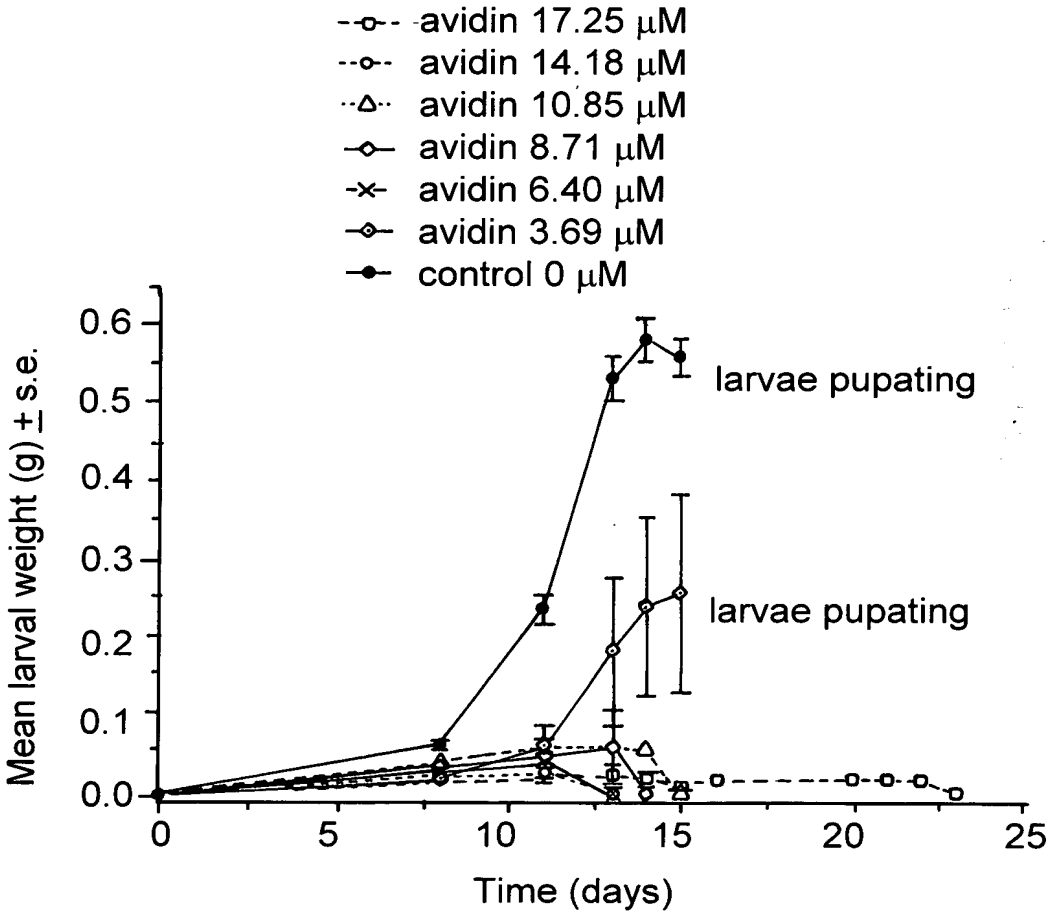


FIG. 25

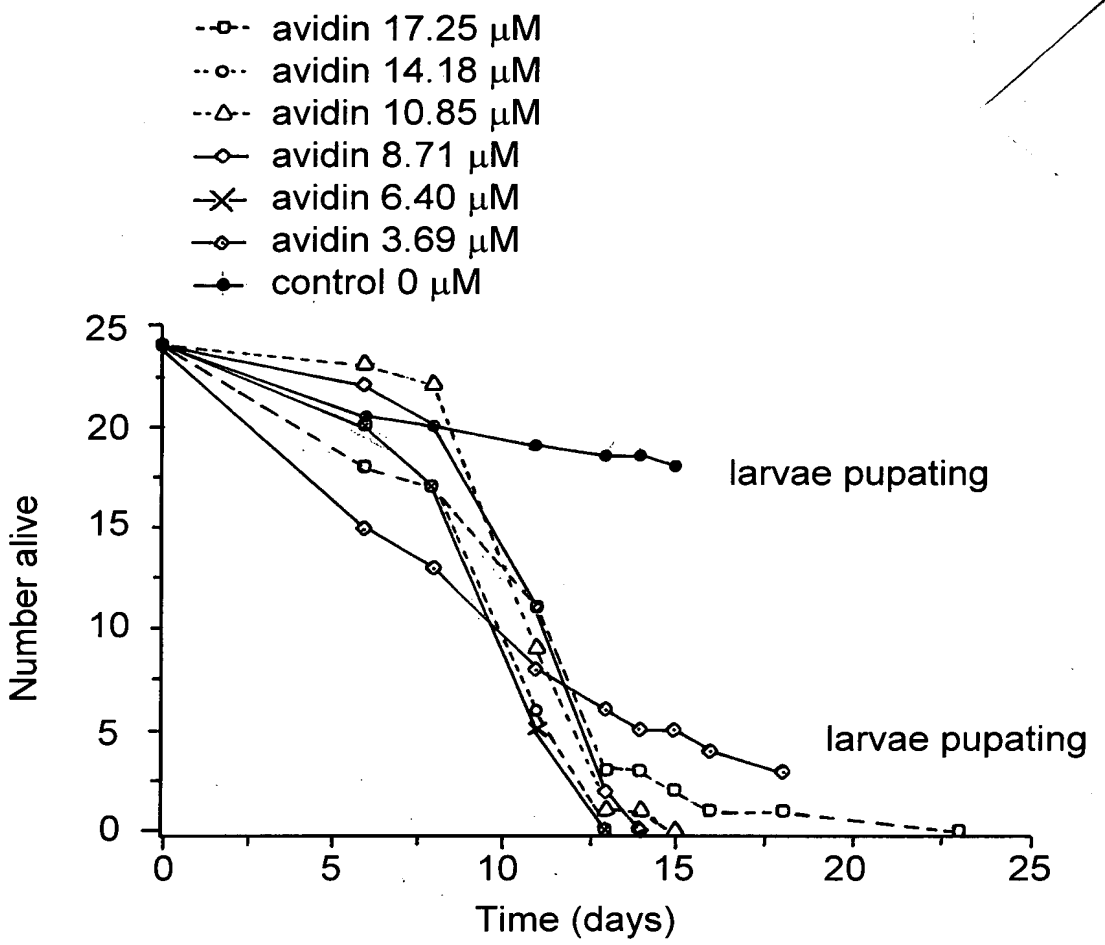


FIG. 26

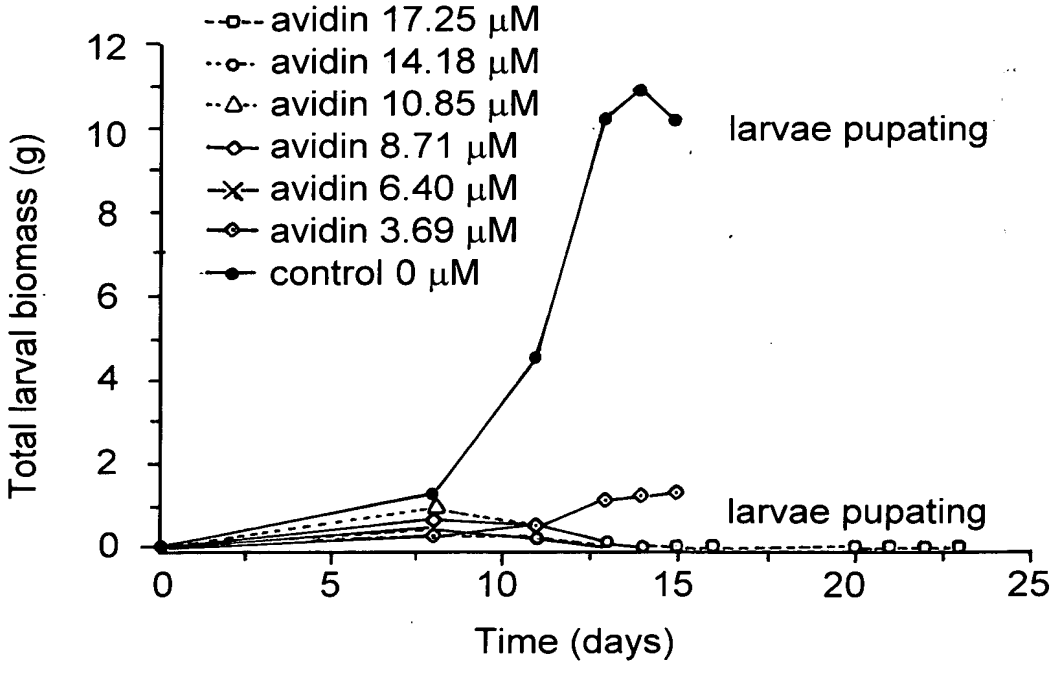


FIG. 27

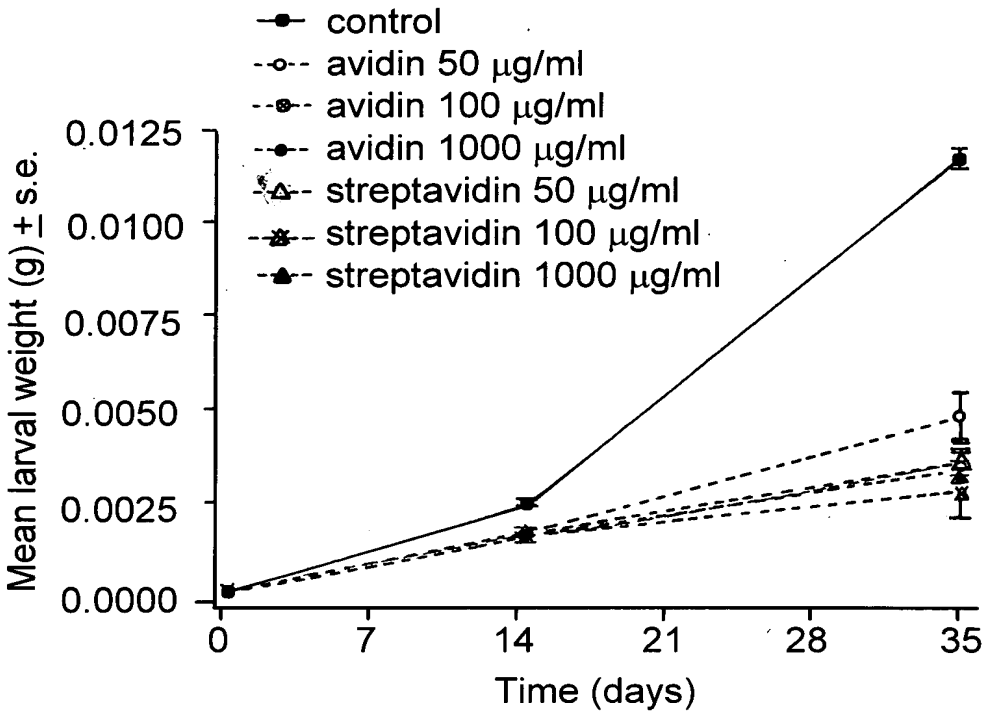


FIG. 28

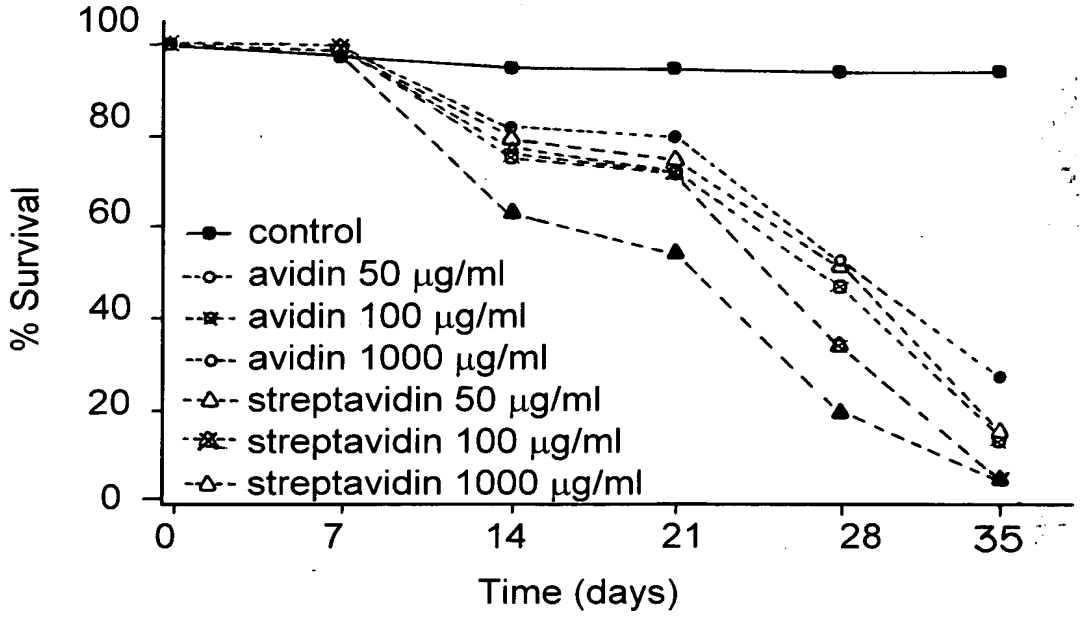


FIG. 29

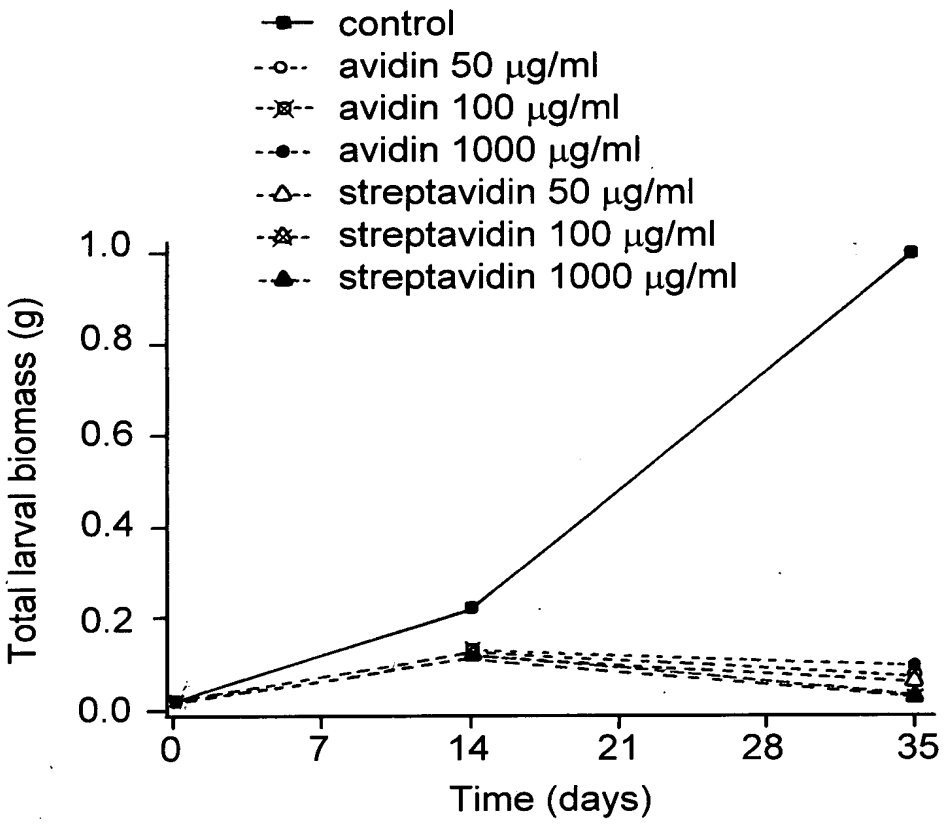


FIG. 30

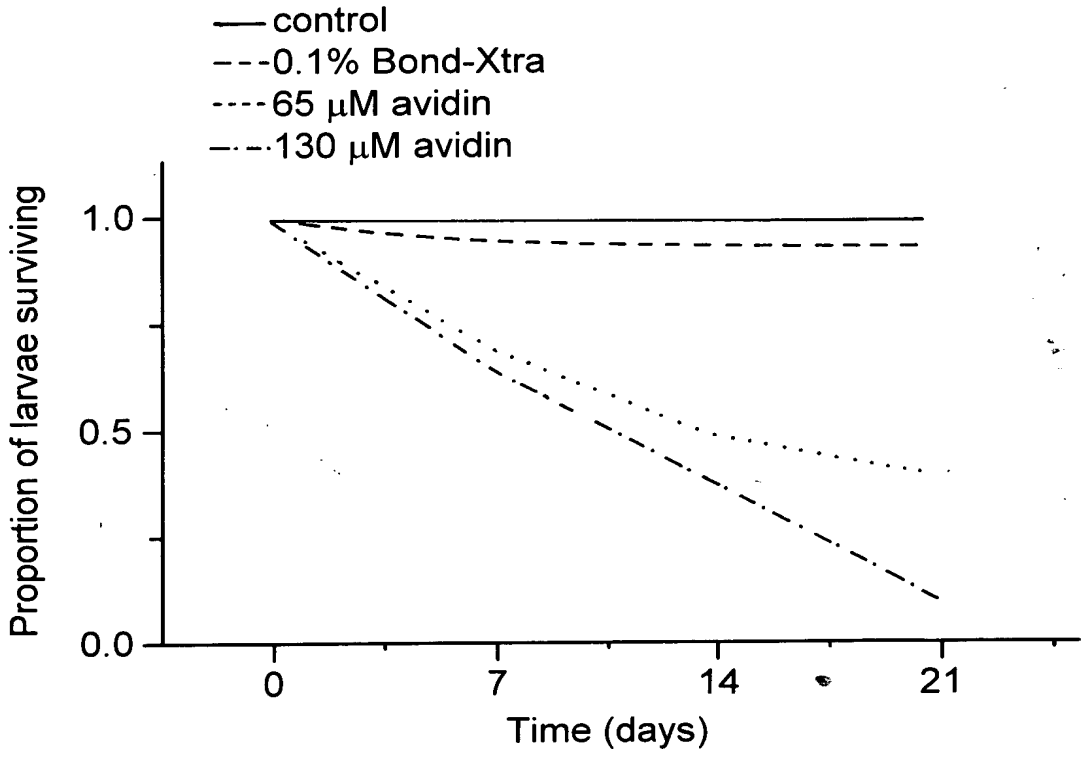


FIG. 31

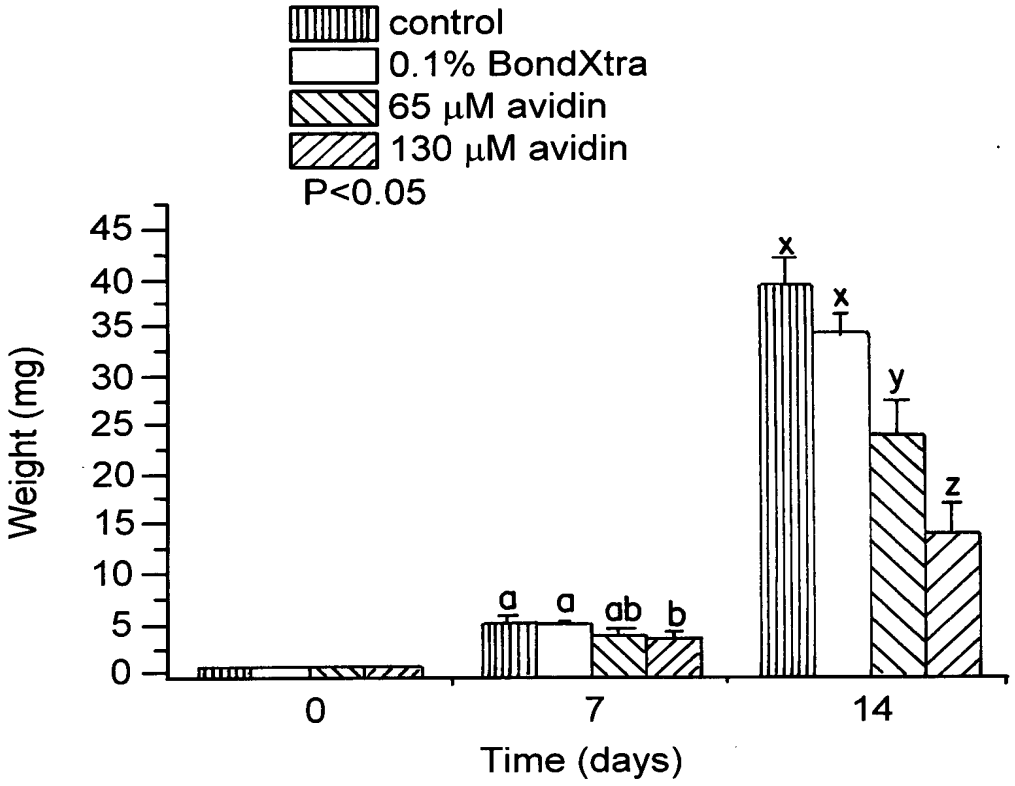


FIG. 32

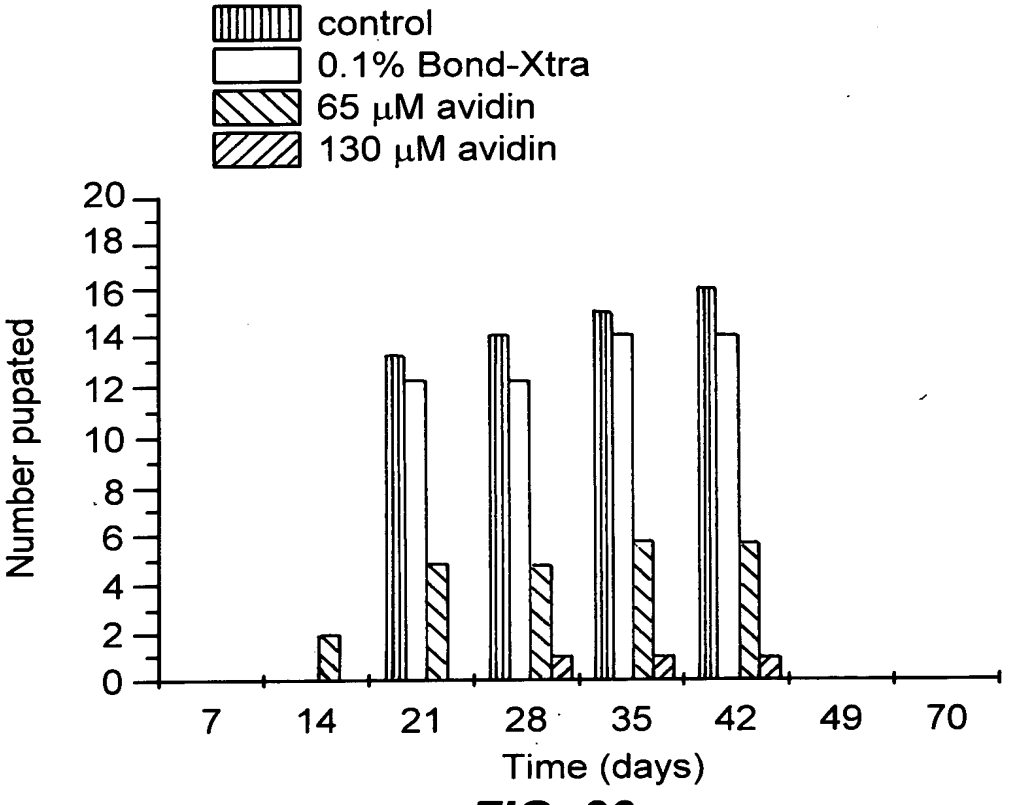


FIG. 33

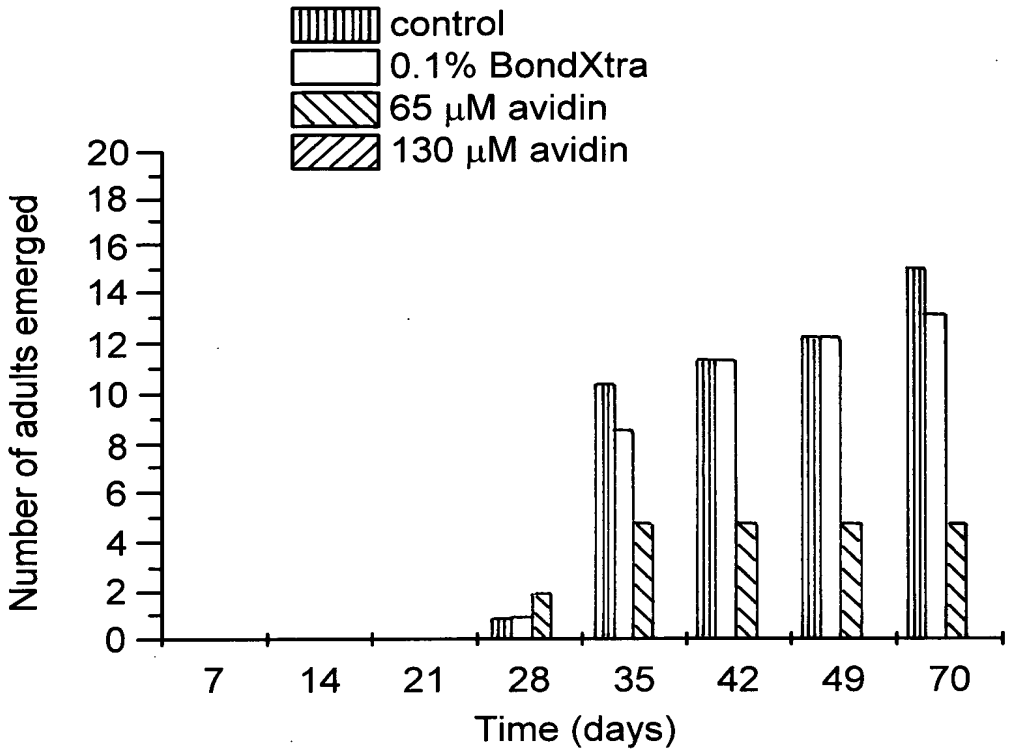


FIG. 34

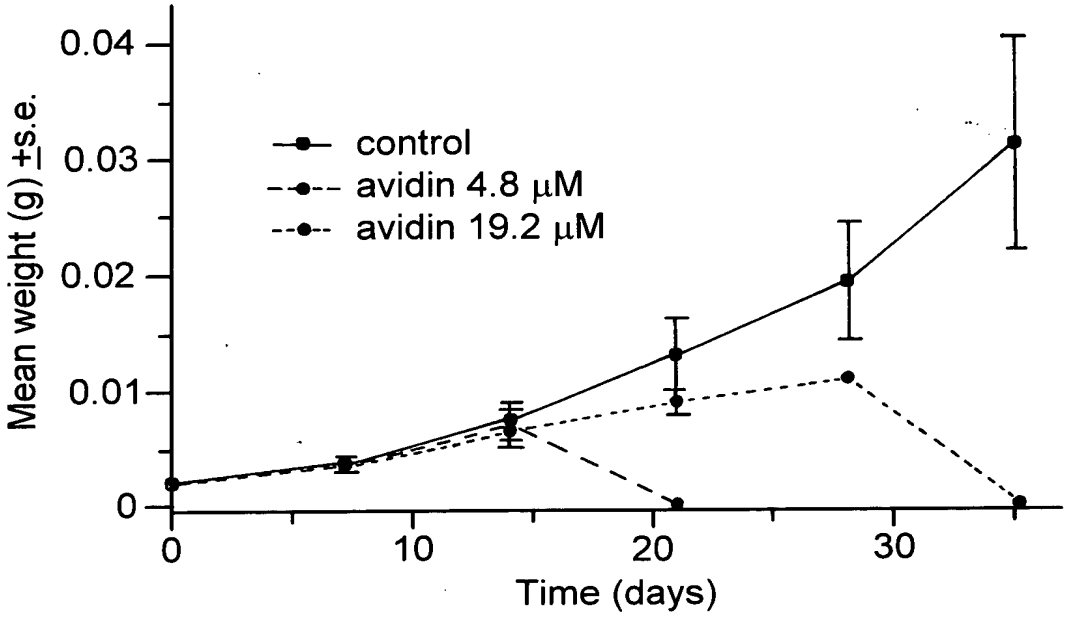


FIG. 35

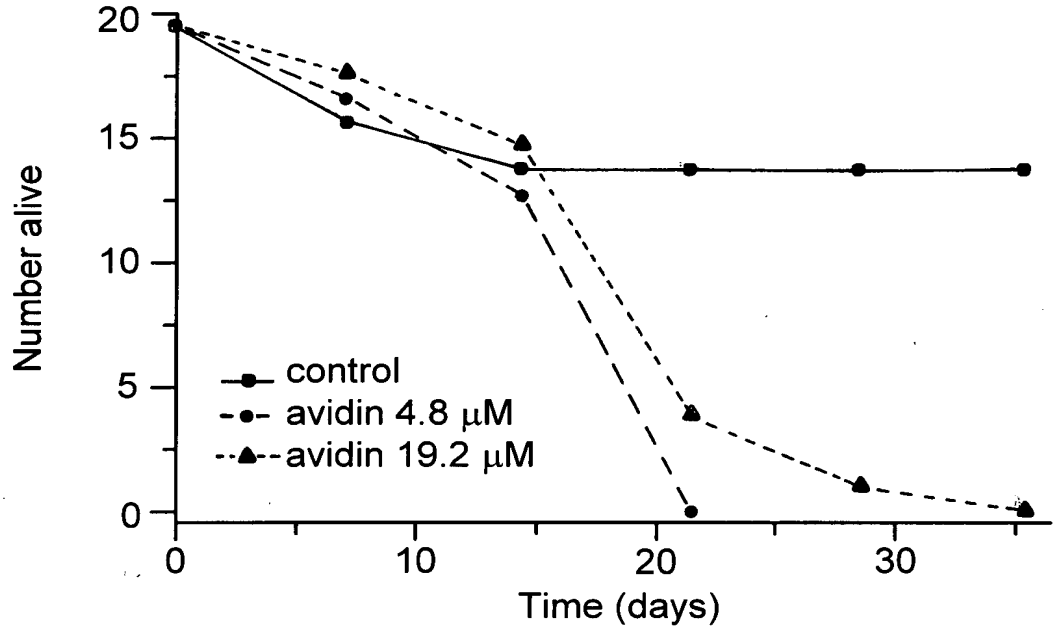


FIG. 36

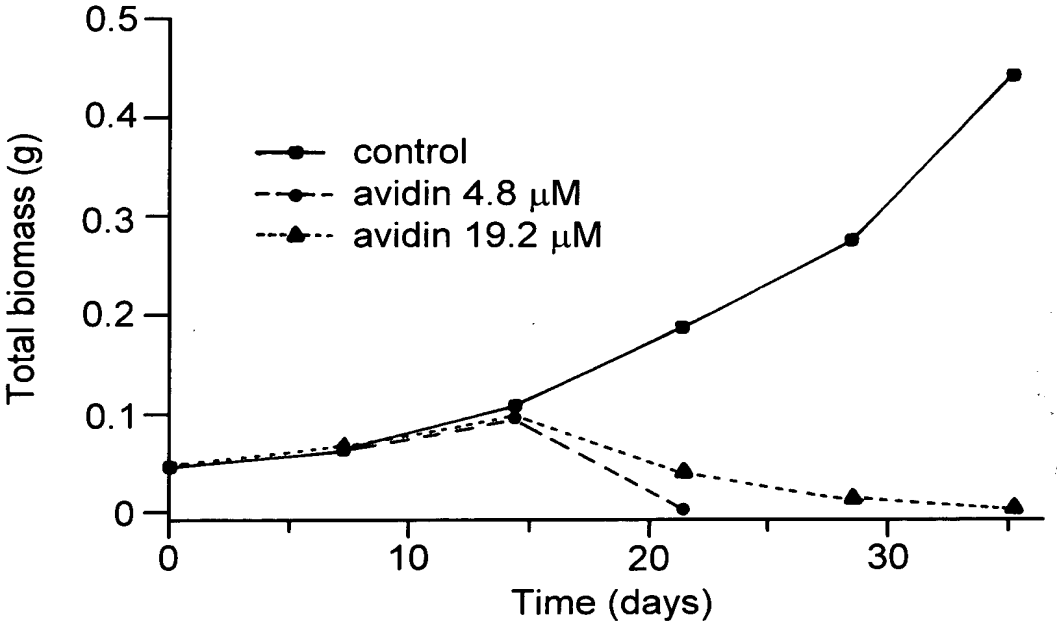


FIG. 37

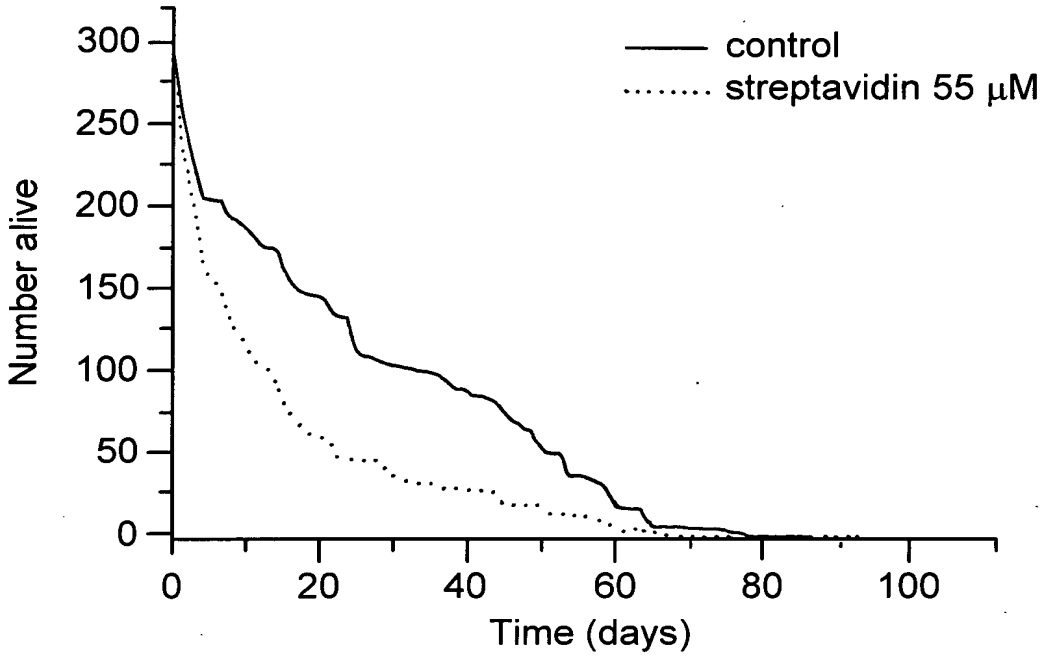


FIG. 38

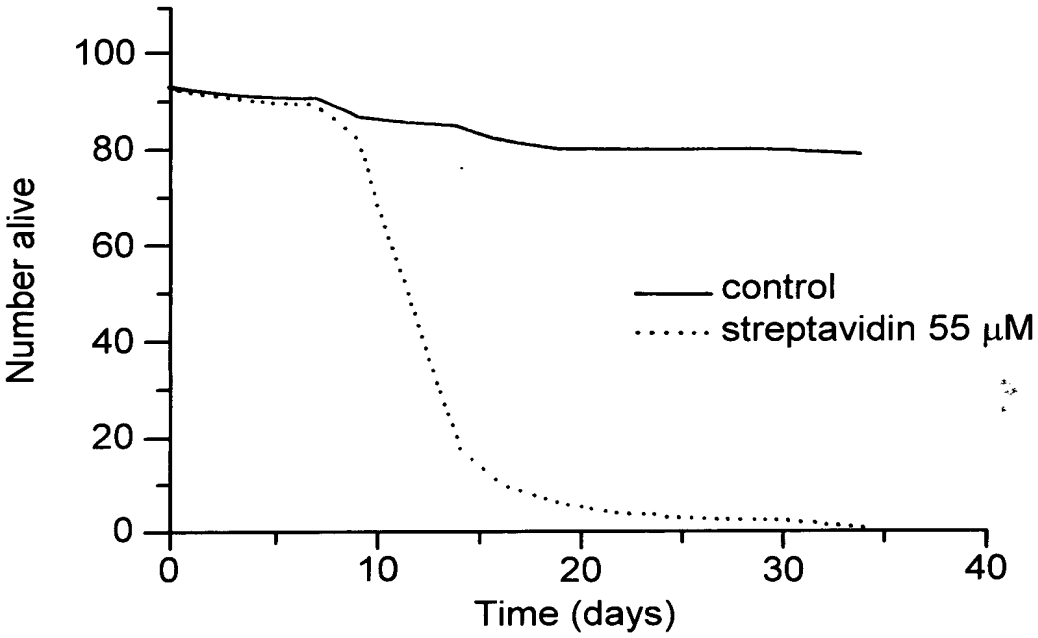


FIG. 39

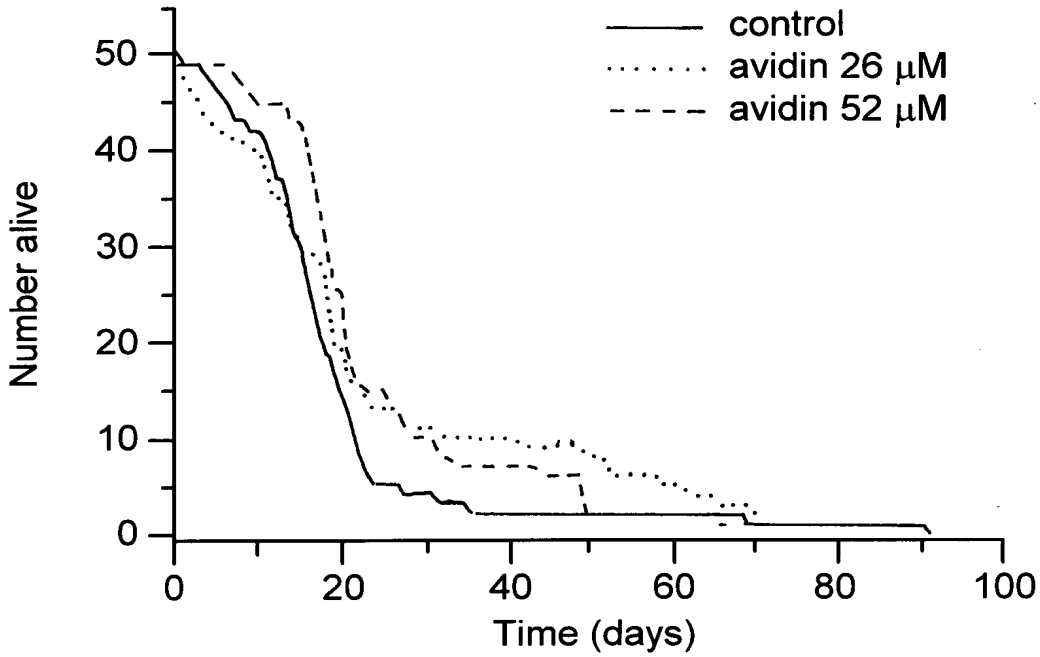


FIG. 40

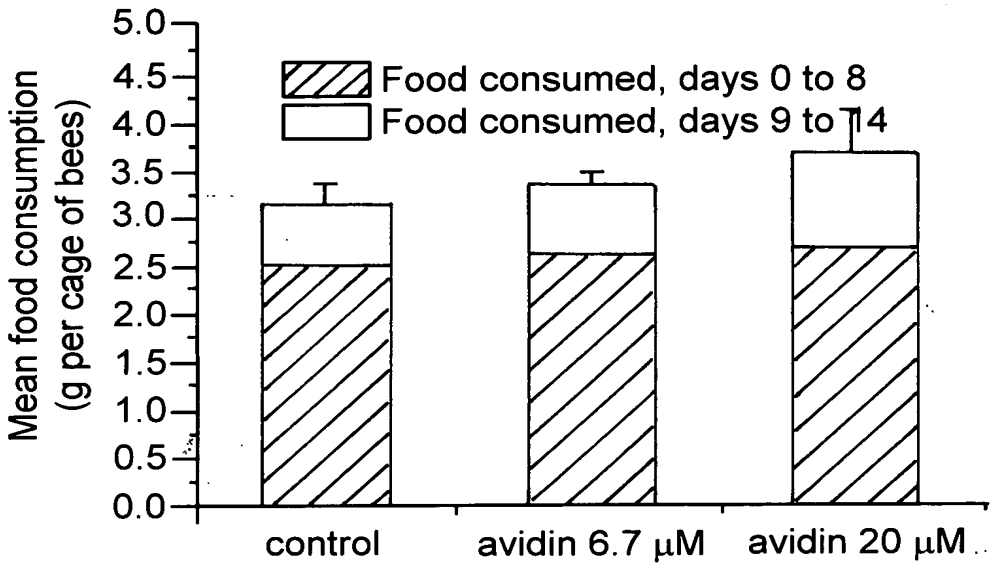


FIG. 41

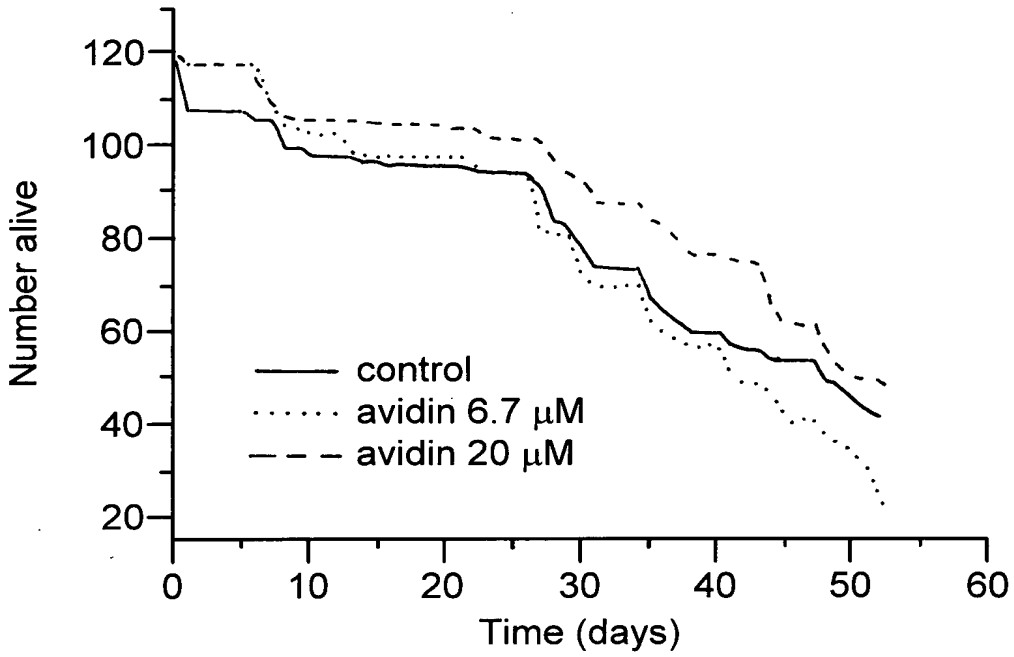


FIG. 42

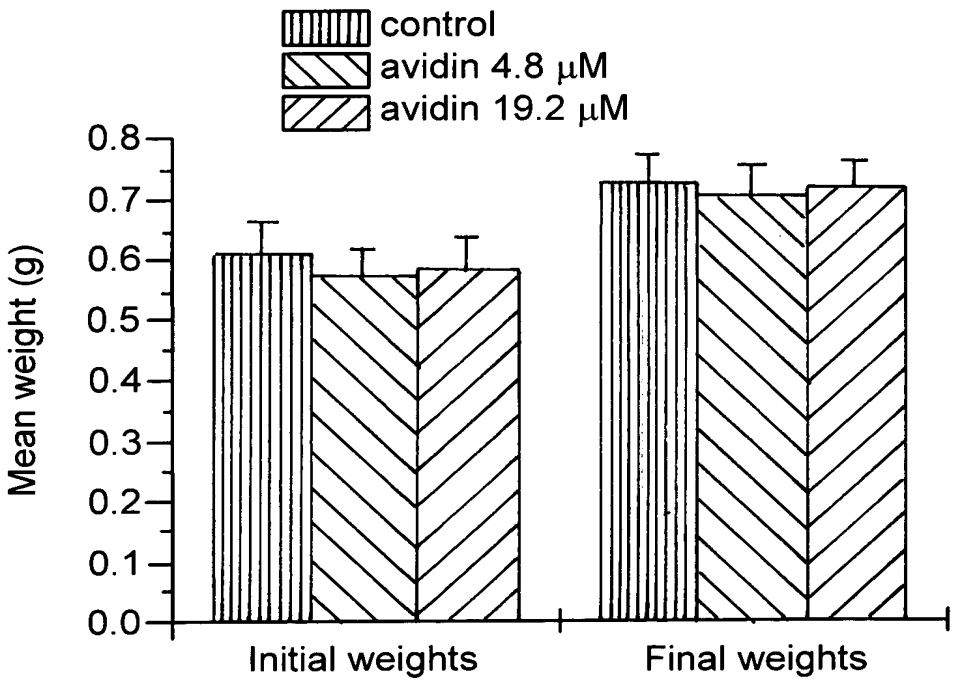


FIG. 43

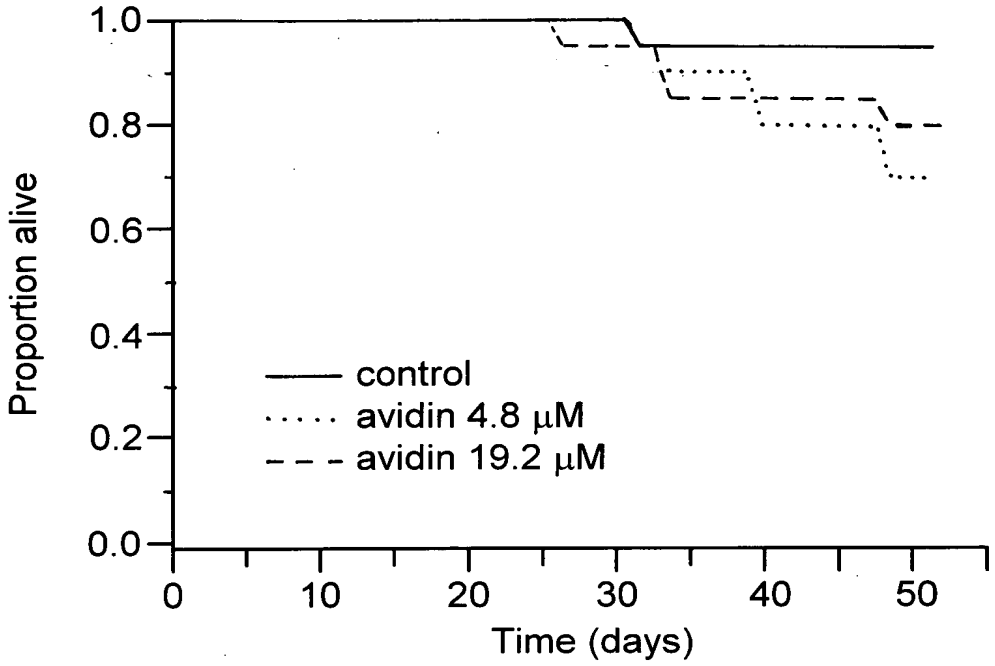


FIG. 44

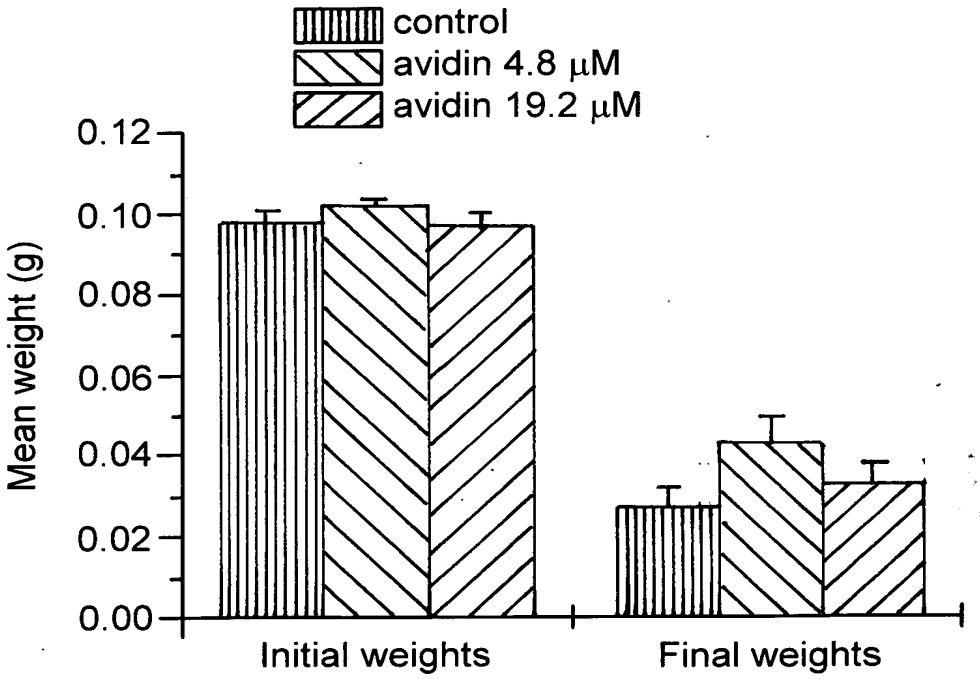


FIG. 45

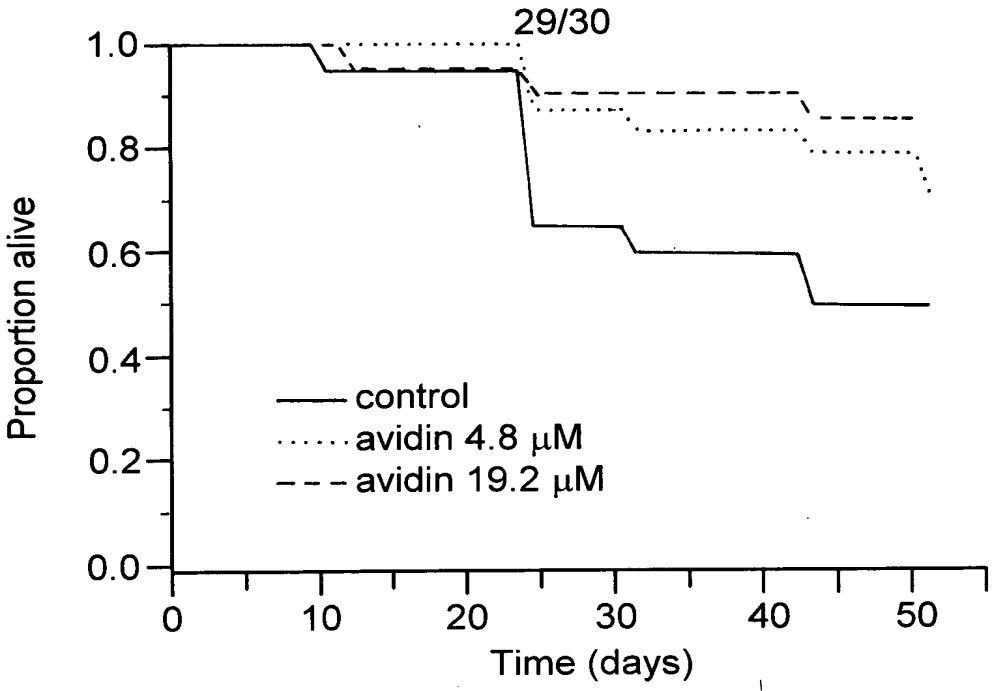


FIG. 46

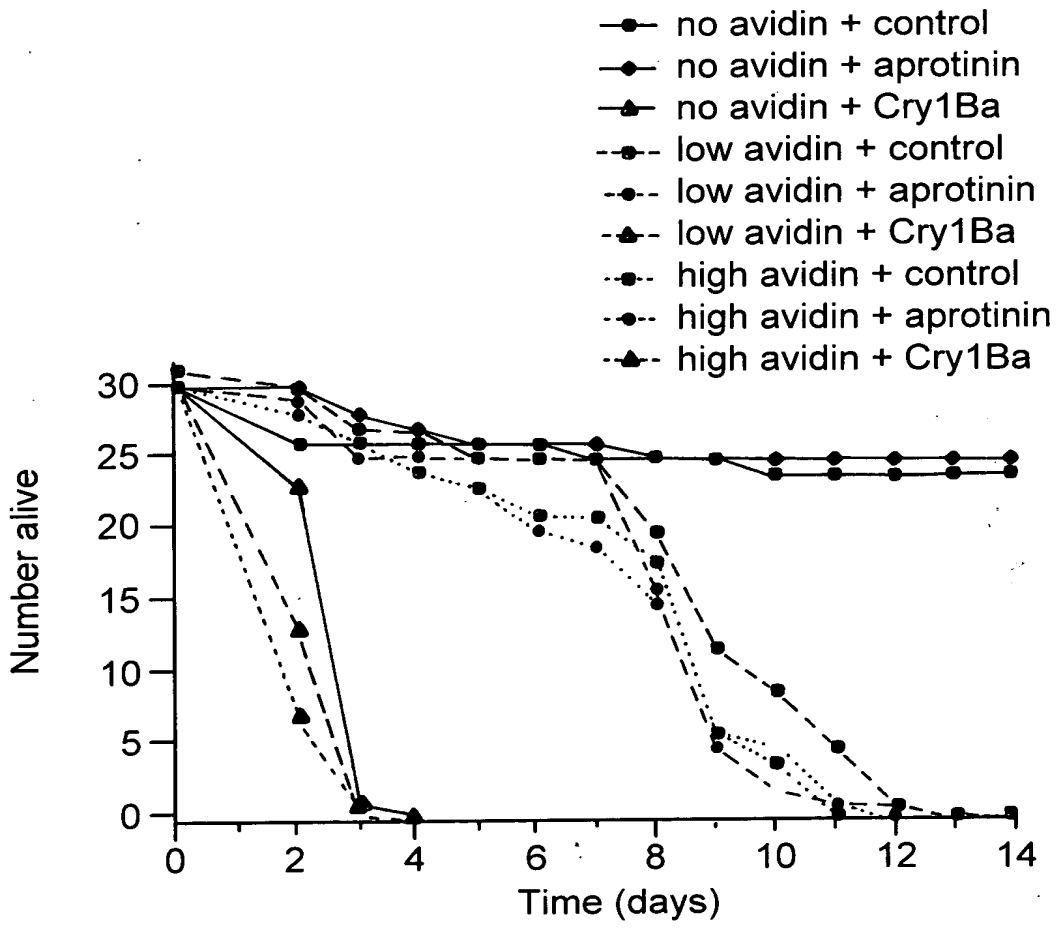


FIG. 47

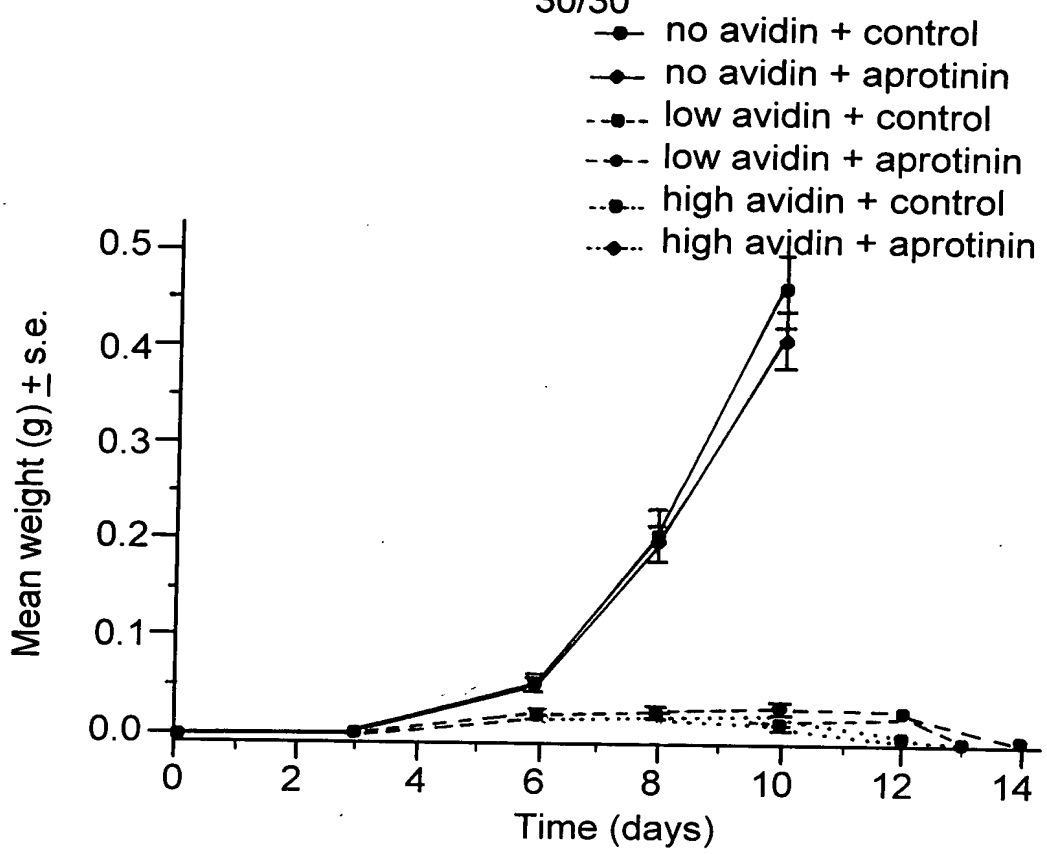


FIG. 48

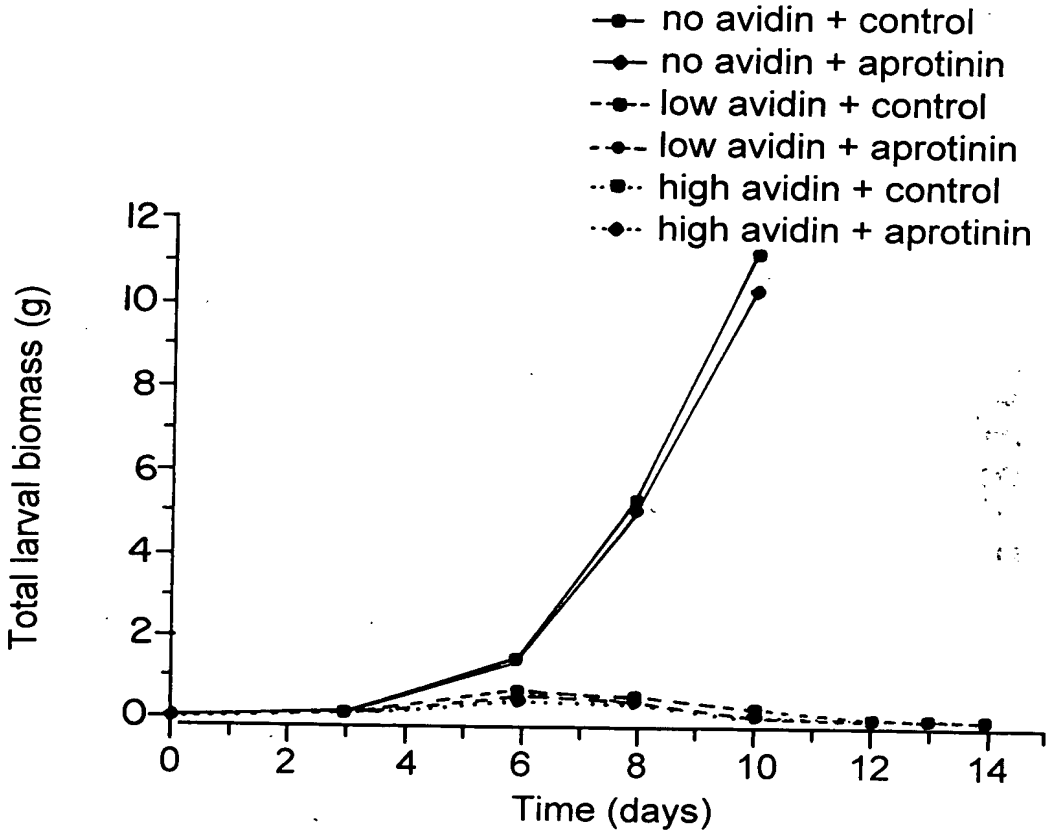


FIG. 49